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1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

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7. All claims of therapeutic efficacy should be based upon explicit comparison with a specific control group, except in special circumstances where each patient is his own control. If nonrandomized controls are used, the characteristics of the patients should be presented in detail and compared to those of the experimental group. Potential sources of bias should be adequately discussed. Comparison of survival between responders and nonresponders does not establish efficacy and should not generally be included. Reports of phase II trials which draw conclusions about antitumor activity but not therapeutic efficacy generally do not require a control group.

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

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* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

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Role of ^{99m}Tc Methylene Diphosphonate Bone Imaging in the Management of Lymphoma[1]

Kenneth C. Anderson,* William D. Kaplan, Robert C. F. Leonard, Arthur T. Skarin, and George P. Canellos[2]

The role of modern techniques of ^{99m}Tc methylene diphosphonate bone imaging in the management of lymphoma patients was assessed by comparing results of 107 bone scans in (*a*) 16 patients with Hodgkin's disease, and (*b*) 45 patients with non-Hodgkin's lymphoma to simultaneous radiologic, clinical, and histopathologic features as well as to subsequent disease course. The sensitivity and specificity were both ≥ 0.96 in both Hodgkin's disease and non-Hodgkin's lymphoma and the overall accuracy by site was 98%. The scan proved to be useful in the definition and follow-up of skeletal lymphomatous disease in both symptomatic and asymptomatic patients, and defined abnormalities which were not predicted by either serum alkaline phosphatase activity or the presence of bone marrow involvement. In no patient, however, did the bone scan result by itself alter either initial staging or estimates of extent of disease at the time of relapse. Bone scanning, therefore, cannot be recommended as a screening procedure in patients with lymphoma; rather, this test is best reserved for the definition and follow-up of skeletal metastases in patients with active, concomitant, extraosseous disease. [Cancer Treat Rep 69:1347–1351, 1985]

Previous reports indicate that osseous involvement in both Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) occurs in at least 10%–15% of these patients (1–4), and emphasize the importance of initial and follow-up evaluations for lymphomatous disease in bone. Bone imaging with ^{99m}Tc methylene diphosphonate (MDP) is a safe, simple, and sensitive screening procedure for detecting lymphomatous involvement of bone, and a useful means of following response to treatment (5). Nonetheless, there remains relatively little published information on the merits of bone imaging using ^{99m}Tc MDP and modern imaging systems in patients with HD and NHL. In this study, we have attempted to define the usefulness of ^{99m}Tc MDP Anger camera bone imaging in lymphoma patients by (*a*) comparing the scan results to concomitant radiologic examinations and laboratory parameters, and (*b*) correlating scan findings with the course of the disease. Our efforts were directed not only at the delineation of the sensitivity and specificity of this technique, but also at the definition of the most appropriate use of this test in the management of patients with HD and NHL.

MATERIALS AND METHODS

Sixty-one consecutive patients, 16 with HD and 45 with NHL, were studied. ^{99m}Tc MPD imaging was performed at the time of diagnosis, at the time of relapse, and/or during follow-up examinations. A total of 107 scans were obtained.

The distribution of the 61 patients and 107 scans by histopathological subgroups is shown in table 1. Nodular sclerosing was the most common cell type in the HD group; diffuse histiocytic lymphoma was the most common cell type in the NHL group. Thirty-two scans were performed for staging (17 at the time of initial diagnosis and 15 at the time of relapse), and the remaining 75 scans were done as follow-up examinations (table 2). Thirty-one patients had a single scan either before or after treatment; the remaining 30 patients had multiple scans.

Imaging was performed 3–4 hours following iv injection of 20 mCi of ^{99m}Tc MDP (New England Nuclear Pharmaceuticals, North Billerica, MA) using a large field-of-view, 37 photomultiplier tube Anger camera fitted

[1]Received Oct 2, 1984; revised Mar 4, 1985; accepted Apr 29, 1985.

[2]Divisions of Medical Oncology (*K. C. Anderson, R. C. F. Leonard, A. T. Skarin,* and *G. P. Canellos*) and Nuclear Medicine (*W. D. Kaplan*), Dana-Farber Cancer Institute, and Harvard Medical School, Boston, MA.

* *Reprint reports to:* Kenneth C. Anderson, MD, Division of Medical Oncology, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

TABLE 1.—Distribution of bone scans according to histopathology

Histopathology	No. of— Patients	Scans
HD		
Nodular sclerosing	7	13
Mixed cellular	6	11
Lymphocyte depleted	1	1
Unspecified	2	4
NHL		
Diffuse histiocytic	26	43
Nodular poorly differentiated	4	6
Diffuse poorly differentiated	10	21
Diffuse mixed	3	5
Burkitt's lymphoma	1	1
Unspecified	1	2
Total	61	107

with a parallel hole, low-energy all-purpose collimator (Siemens Corporation, Iselin, NJ). With the patient on a moving bed, anterior and posterior images of the entire body were accumulated (scan speed of 24 cm/min); selected 500,000 count static images of the axial skeleton were routinely obtained.

Clinical data were derived from other radiographic examinations and analysis of the subsequent clinical course. All patients in this series had clinical data acquired and/or at least one other radiologic procedure performed within 3 weeks of the bone scan. A bone scan result was considered true-positive if lymphomatous involvement in bone was documented within a 6-month interval from the date of obtaining an abnormal scan. In contrast, a normal bone scan was considered to be true-negative if bony disease due to lymphoma did not develop within a similar time period from the time when an abnormal scan was obtained. A scan was considered either false-positive or false-negative if it did not correlate with and/or predict the disease course of lymphoma in bone for at least 6 months.

Bone scans were interpreted in a blind fashion with no information on histopathologic subtype, clinical history, or laboratory parameters available to the nuclear medicine physician (W. D. Kaplan) who read the scans. Comparison of scan results with simultaneous radiologic, clinical, laboratory, and histopathologic features, as well as with subsequent disease course, was carried out only after the interpretation of bone scans was completed.

RESULTS

Implications of a Normal Bone Scan

Fifty-nine scans in 41 patients were interpreted as normal; there were no false-negative scans (table 3). Nine scans were performed at the time of initial staging when six patients had stage IV disease and one patient each had stage I, II, and III disease. Symptoms of bone pain were present at the time of scanning in only nine of the 59 (15%) normal bone scans (table 4). Bone disease did not subsequently develop in any of these symptomatic patients. Comparison radiologic scans, performed within 3 weeks of scan, were available for 36 scans in 24 patients and were uniformly normal. No patient evidenced an abnormal radiograph and normal scan. Fifty-nine serum alkaline phosphatase determinations and 32 bone marrow examinations were done at the time of the 59 normal bone scans. Serum alkaline phosphatase was elevated at the time of 17 of 59 (29%) normal scans. At the time of imaging, bone marrow was involved by lymphoma in eight of 32 (25%) normal scans, more commonly in NHL (32%) than in HD (10%).

TABLE 2.—Distribution of bone scans according to treatment status (107 scans in 61 patients)

Treatment status	No. of patients (No. of scans) HD	NHL
Initial pretreatment scan (staging)*	2 (2)	15 (15)
Post-initial treatment	9 (13)	24 (39)
At relapse	5 (5)	9 (10)
Post-relapse treatment	7 (9)	11 (14)
Total	(29)	(78)

*Stage I, 1 patient; stage II, 2; stage III or IV, 14.

Relevance of an Abnormal Bone Scan

Forty-eight scans in 24 patients revealed bone disease, including two false-positive scans (table 3). Eight scans were performed at the time of initial staging when seven patients had stage III or IV disease and a single patient had stage II disease. The abnormal bone scan result by itself did not alter initial stage of disease in any patient. The remaining 40 abnormal scans were obtained either at the time of relapse or routine follow-up. In no case was the bone scan the sole indicator of recurrent or advanced disease. Ten scans were abnormal at a single site and 38 scans were abnormal at multiple sites. Symptoms of bone

TABLE 3.—Bone scan correlation with subsequent disease course

Disease	No. of patients (No. of scans) Bone scan normal	Bone scan abnormal
HD		
Bone disease present	0 (0)	7 (14)
Bone disease absent	11 (15)	0 (0)
NHL		
Bone disease present	0 (0)	15 (32)
Bone disease absent	30 (44)	2* (2*)
HD + NHL		
Bone disease present	0 (0)	22 (46)
Bone disease absent	41 (59)	2* (2*)

*False-positive scans: both abnormal at a single site.

pain at the site of scan abnormality were present at the time of 23 of the 48 (48%) scans (table 4). Concomitant (within 3 weeks) radiologic reports were available for correlation with bone scan data in all cases where bone scans were interpreted as revealing metastatic disease. Radiographs were abnormal in 31 (65%) cases, but in 17 (35%) cases, the abnormal bone scan was the sole indicator of bone disease. Forty-seven serum alkaline phosphatase assays and 18 bone marrow examinations were performed at the time of the 48 abnormal scans. The serum alkaline phosphatase was elevated in 19 of 47 (40%) patients and bone marrow involvement was documented in only two of 18 (11%) patients.

Clinical Correlations

The accuracy of ^{99m}Tc MDP bone scanning in defining or excluding sites of active skeletal lymphoma was 98%, with a sensitivity and specificity of ≥ 0.96 in patients with HD and NHL (table 5). Median follow-up was 26 months from the time of initial scanning (range, 6–70).

There were no false-negative bone scans and two false-positive bone scan examinations (table 3). The first of these occurred in a 58-year-old white male with stage IVB diffuse mixed lymphocytic and histiocytic lymphoma whose scan revealed focal uptake in the right sacrum compatible with metastatic disease. No evidence of disease has developed at these sites 5 years after his abnormal scan. The second false-positive scan was obtained in a 54-year-old white woman with diffuse histiocytic lymphoma in complete remission whose scan revealed focal increased uptake in the left femoral neck, raising the suspicion of metastatic disease. She remains without any evidence of active disease 3 years later.

DISCUSSION

Our data show that ^{99m}Tc MDP imaging using an Anger camera provides a highly accurate examination for the definition and follow-up of bone disease in patients with HD and NHL. In our series, the false-negative rate was zero and the false-positive rate was only 2% overall (two of 107 scans). Arthritis, a potential source of false-positive scans, was correctly identified by the pattern of tracer uptake in all cases. Therefore, the overall accuracy was excellent (98%).

Bone involvement with either HD or NHL has been reported in 10%–15% of patients at some time during their disease course (1–4). Since treatment is planned according to stage of disease, it would seem important to confirm bone involvement in these patients. Skeletal radiography is associated with a high rate of false-negative results (6), in contrast to bone imaging using ^{99m}Tc MDP, which has been useful in defining bone disease in various malignancies including HD and NHL (5,7–15). Six of 17 (5), two of ten (7), and 17 of 48 radiographs (our series) acquired in lymphoma patients at the time of abnormal bone scans were normal. The abnormal scan, therefore, was not only more sensitive than radiographs, but in these patients it reflected the only evidence of metastatic disease in bone.

As is well known, ^{99m}Tc MDP imaging can yield false-negative results due to either increased, but uniform and symmetric, tracer uptake from disseminated skeletal metastases, or in cases of pure osteolytic lesions (15,16). In our series of 107 scans, no false-negative scans were noted. Patients were instructed to void immediately prior to scanning, thereby avoiding the potential obscuration of a pelvic lesion secondary to overlying bladder activity. Moreover, permeative osteolytic lesions or related pathologic fractures were not documented in any of our patients.

^{99m}Tc MDP bone imaging has also been criticized in reports of false-positive scans. Spencer and Datu (17), for instance, have recorded 11 common, 14 uncommon, and ten rare causes of bilateral lower limb uptake of bone scanning tracers, emphasizing the importance of precisely defining the etiology of abnormally increased tracer

TABLE 4.—Bone scan result correlated with clinical parameters at time of imaging

Clinical parameters	HD		NHL		HD + NHL	
	No. of scans/ total No. (%)	No. of patients/ total No. (%)	No. of scans/ total No. (%)	No. of patients/ total No. (%)	No. of scans/ total No. (%)	No. of patients/ total No. (%)
Bone scan normal						
Bone pain	2/15 (13)	2/11 (18)	7/44 (16)	7/30 (23)	9/59 (15)	9/41 (22)
Abnormal radiograph	0/12 (0)	0/8 (0)	0/24 (0)	0/16 (0)	0/36 (0)	0/24 (0)
Elevated serum alkaline phosphatase	6/15 (40)	5/11 (45)	11/44 (25)	11/30 (37)	17/59 (29)	16/41 (39)
Bone marrow involvement	1/10 (10)	1/8 (13)	7/22 (32)	3/14 (21)	8/32 (25)	4/22 (18)
Bone scan abnormal						
Bone pain	6/14 (43)	4/7 (57)	17/34 (50)	13/17 (76)	23/48 (48)	17/24 (71)
Abnormal radiograph	10/14 (71)	5/7 (71)	21/34 (62)	12/17 (71)	31/48 (65)	17/24 (71)
Elevated serum alkaline phosphatase	8/14 (57)	5/7 (71)	11/34 (33)	8/17 (47)	19/48 (40)	13/24 (54)
Bone marrow involvement	1/5 (20)	1/5 (20)	1/13 (8)	1/11 (9)	2/18 (11)	2/16 (13)

TABLE 5.—Specificity and sensitivity of bone scans

NHL	
Specificity*	0.96
Sensitivity†	1.0
HD	
Specificity	1.0
Sensitivity	1.0
NHL + HD	
Accuracy‡	0.98

*Specificity (true-negative/true-negative and false-positive).

†Sensitivity (true-positive/true-positive and false-negative).

‡Accuracy (true-positive and true-negative/true-positive and true-negative and false-positive and false-negative).

uptake on bone scan. A recent report of unilateral vertebral arch hypertrophy causing bone scan abnormality in a patient with Hodgkin's disease (18) further stresses the importance of a diligent search for the cause of each bone scan abnormality by correlating scan findings with bone x-rays, history, and when indicated, linear or computed tomography (19). In our series, only two scans with focal increased uptake were judged to be falsely positive, since these patients did not subsequently develop disease over the next 3–5 years. Since bone disease as defined by an abnormal scan never occurred in our patients without evidence of active extraskeletal lymphoma, it seems particularly important to precisely define the cause of bone scan abnormality in patients with HD and NHL who are otherwise without active disease, as spread of lymphoma in bone is unlikely in this setting.

Many reports have attempted to correlate either clinical findings (ie, presence or absence of pain) or laboratory parameters (ie, serum alkaline phosphatase activity or positive bone marrow histology) with scan results. For example, Brady and Croll (10) have reported that 73% of general cancer patients with bone-related symptoms have abnormal bone scans, whereas 45% of patients without pain have abnormal scans. Schechter et al (5) also noted abnormal scans with greatest frequency in those lymphoma patients who had bone pain (11 of 11). In our series, bone pain was similarly more common when scans were abnormal (23 of 48 patients) than normal (nine of 59) (table 4). Nonetheless, it is important to emphasize that fully one-half of our abnormal scans occurred in asymptomatic patients, supporting and underscoring the concept that symptoms do not always correlate with scan results. Serum alkaline phosphatase, reportedly elevated at the time of five of six abnormal scans in lymphoma patients (5), was elevated at the time that 40% of the abnormal scans and 29% of the normal scans were performed in our series, which indicates that this parameter is also of little predictive or discriminative value for skeletal involvement by lymphoma. Bone marrow involvement has been documented at the time of 11 of 16 (69%) abnormal scans in lymphoma patients (5); in contrast, in our series only two of 18 (11%) patients with abnormal scans had bone marrow involvement whereas eight of 32 (25%) patients with normal scans had documented marrow disease. This probably reflects the fact that five of our eight patients with bone marrow involvement and normal scans had diffuse poorly differentiated lymphoma, a commonly systemic disease. This also suggests that an abnormality on scan is not predictive of tumor in the bone marrow. Finally, in contrast to the report of Schechter et al (5), in our series the bone scan was not more commonly abnormal in more advanced stages of disease. Thus, in our experience, results of bone imaging in patients with lymphoma do not appear to correlate with either symptoms or other laboratory parameters and do provide a means of obtaining unique clinical information.

Although there may be some selection bias in our study, it is important to note that in patients with HD and NHL, the percentage of scans with comparison data available for the presence of bone pain, abnormal radiograph, elevated serum alkaline phosphatase, and bone marrow involvement is very similar. Assessment of histologic subtype, the presence of bone pain, or an elevated serum alkaline phosphatase was available at the time of all scans (table 4). Radiographs were available for comparison with all abnormal scans and 36 of 59 (61%) normal scans. Assessment of bone marrow involvement was available for 32 of 59 (54%) abnormal scans and 18 of 48 (38%) normal scans. If there was a selection bias, the similar numbers of data points available for each of these parameters within the normal and abnormal bone scan groups appear to allow a relative comparison of the incidence of these various parameters in the two major normal and abnormal scan groups. Moreover, the bone scans were interpreted in a blind fashion with no information on histopathologic subtype, clinical history, or laboratory parameters available to the nuclear medicine physician who read the scans. Comparison of scan results with simultaneous radiologic, clinical, and histopathologic features, as well as with subsequent disease course, was carried out only after interpretation of bone scans was completed.

In summary, our data show that ^{99m}Tc MDP imaging in HD and NHL is highly specific and sensitive and therefore has high overall accuracy in the definition and follow-up of lymphomatous disease to bone. The presence of bone-related symptoms, elevated serum alkaline phosphatase, bone marrow involvement, or advanced stage of disease did not either predict for, or correlate with, scan result, and, in fact, 35% of the abnormal scans were the sole indicator of lymphomatous spread to bone. Although ^{99m}Tc MDP imaging therefore provided information not anticipated by symptoms or other laboratory parameters, it is important to emphasize that the scan result by itself did not alter initial stage of disease or therapeutic strategy in any patient. Moreover, while extremely

useful for defining bone disease and following the response of bone to treatment, an abnormal scan was never the sole indicator of active disease at the time of relapse. ^{99m}Tc MDP imaging in patients with HD and NHL would, therefore, appear to be a sensitive means of following lymphomatous disease in bone but will uncommonly define bone as the sole site of active disease, especially in the setting of relapsed HD or NHL.

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Antiemetic Activity of Two Different High Doses of Metoclopramide in Cisplatin-Treated Cancer Patients: A Randomized Double-Blind Trial of the Italian Oncology Group for Clinical Research[1,2]

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High-dose metoclopramide has been proposed as an effective antiemetic agent in patients treated with cisplatin. Its optimal dosage schedule, however, has not yet been completely defined. We report the results of a multicenter, double-blind, randomized clinical trial where the efficacy and safety of two fixed high-dose regimens of metoclopramide (60 and 120 mg, respectively) have been tested in 112 patients receiving cisplatin treatment. No statistically significant difference has been found between the two groups. In patients treated with cisplatin at doses > 100 mg/m^2, the higher dose regimen seems more efficacious, but this result should be confirmed in a larger group of patients. Treatment was generally well-tolerated. However, a consistent percentage of patients (about 60%) still present with vomiting, and this makes further investigation necessary. [Cancer Treat Rep 69:1353–1357, 1985]

The severe nausea and vomiting induced by several active cancer chemotherapeutic agents is often the most distressing side effect of treatment, which can also frequently prevent completion of a potentially curative therapy program (1). Cisplatin is a highly emetic cytotoxic agent, with vomiting being recorded in > 70% of the treated patients (2), although a high degree of variability according to dosages and administration modalities has been reported (3). High-dose metoclopramide (MTC) (1–3 mg/kg iv) has been proposed as the most effective antiemetic agent in these patients (4). However, its efficacy and safety profile as well as the optimal dosage schedule appear to vary widely in different studies (5–14). These uncertainties are possibly due to the inappropriate design of the studies, the different dosages used, and the small number of patients treated (those who have often already undergone other chemotherapeutic courses and who were receiving combination therapies).

The aim of the study was, therefore, to analyze the efficacy and toxicity of two fixed high-dose regimens of MTC in patients treated with cisplatin for the first time in a multicenter, double-blind, randomized clinical trial.

PATIENTS AND METHODS

From December 1983 to May 1984, all previously untreated patients with a performance status ≥ 60 (Karnofsky) and scheduled for chemotherapeutic treatments with ≥ 50 mg/m^2 of cisplatin were considered eligible for the study. When cisplatin was used in combination with other cytotoxic drugs, it was administered on Day 1 and the other drugs were administered 24 hours later, after completion of the evaluation of the antiemetic effect. Nausea and vomiting from other causes, cerebral metastases, radiotherapy, and use of narcotic analgesics and CNS sedatives were considered criteria for exclusion. The standard prerequisites for admission to chemotherapy protocols (wbc count ≥ 4000/mm^3, platelet count ≥ 120,000/mm^3, serum creatinine ≤ 1.5 mg/100 ml, and

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serum profile for electrolytes and enzymes) were observed.

After obtaining fully informed consent, patients were randomly assigned to one of two MTC regimens, either the 60-mg or the 120-mg pro dose. Each dose was diluted in 100 ml of saline and administered as a 15-minute infusion at 30 minutes before and at 90, 210, and 330 minutes after cisplatin, for a total dose of 240 or 480 mg, respectively. Blindness was assured, since the randomly assigned blocks of solutions were known only to the hospital pharmacist who prepared them. Cisplatin treatment followed the usual iv hydration. Mannitol (12.5 g) was given iv in 30 minutes immediately before cisplatin administration. Patients were allowed to have their normal intake of food. No other antiemetics were administered during the 24-hour observation period. Efficacy assessment of antiemetic treatment was based on the overall count of the vomiting episodes. Complete protection was defined as the absence of vomiting; major protection was defined as less than or equal to two vomiting episodes; minor protection was defined as three to five vomiting episodes; and failure of treatment was defined as more than five vomiting episodes. The evaluation of the intensity of nausea was monitored at 2, 4, 6, 8, and 24 hours after chemotherapy and was performed on the basis of both a score system and a linear analog scale. The score was calculated by the attending personnel and decided after questioning of the patient, according to the following scale: 0 = no nausea; 1 = slight nausea; 2 = moderate nausea; and 3 = severe nausea. The patient was then requested to record the degree of nausea by pointing to a linear analog scale. With this method we arbitrarily considered slight nausea as corresponding to a mark of < 40 mm, moderate nausea as corresponding to a mark of between 40 and 69 mm, and severe nausea as corresponding to a mark of > 70 mm.

Side effects were also assessed in detail by general questioning and monitoring of the patient at the same above-mentioned intervals. The evaluation of antiemetic treatment was performed on the first and only course of cisplatin.

Statistical Analysis

A Mann-Whitney U test was used for the significance of differences between the mean values of the dependent variables (number of vomiting episodes, score of maximal nausea, and maximal linear visual analog). Four-way ANOVA models were performed to evaluate the significance of differences among the means of each dependent variable with respect to the levels of the following factors: sex [male (M), female (F)]; age [$<$ 50 years old (Y), 50–64 years old (M), and $\geq$ 65 years old (O)]; dose of cisplatin [$<$ 80 mg/m^2 (L), 80–99 mg/m^2 (M), and $\geq$ 100 mg/m^2 (H)]; and dose of MTC [60 mg (l) and 120 mg (h)].

Generalized linear models were used to fit the data. More precisely, logit-linear models were fitted when dependent variables were presence of vomiting and nausea, log-linear models for score of nausea and for number of vomiting episodes (for the latter, a model with the interaction between cisplatin and MTC doses was chosen), and linear models for the linear visual analog. All P values correspond to a two-tailed significance test.

RESULTS

A total of 134 patients were entered in the study. Twenty-two of the patients were not evaluable for response for the following reasons: doses of cisplatin administered after recalculation were actually inferior to 50 mg/m^2 (17 patients), error in randomization (one) or in antiemetic treatment (one), and early toxic effects (three). All 134 patients were evaluable for toxicity.

The characteristics of the 112 evaluable patients are shown in table 1, which documents the satisfactory efficiency of the randomization design with respect to the

TABLE 1.—Patient characteristics

Characteristic	MTC (60 mg) No. of patients	%	MTC (120 mg) No. of patients	%
Total	59	52.7	53	47.3
Age in yrs*				
31–49	7	11.9	9	17.0
50–64	43	72.9	33	62.3
65–74	9	15.2	11	20.7
Sex				
Men	39	66.1	35	66.0
Women	20	33.9	18	34.0
Performance status				
90–100	30	50.8	24	45.3
60–80	29	49.2	29	54.7
Type of cancer				
Lung carcinoma				
Small cell	10	16.9	9	17.0
Non-small cell	19	32.2	13	24.5
Ovarian carcioma	14	23.7	8	15.1
Head and neck squamous carcinoma	7	11.9	6	11.3
Prostate carcinoma	3	15.3	2	32.1
Esophageal-gastric carcinoma	0		4	
Bladder carcinoma	2		3	
Breast carcinoma	2		1	
Other†	2		7	
Dose of cisplatin (mg/m^2)				
50–79	38	64.4	38	71.7
80–99	11	18.6	8	15.1
$\geq$ 100	10	17.0	7	13.2

*Average, 57.36 for the 60-mg dose; average, 56.98 for the 120-mg dose.

†*60-mg group*—uterus carcinoma (1 patient) and melanoma (1); *120-mg group*—Hodgkin's lymphoma (1 patient); anus carcinoma (1); rhabdomyosarcoma (1); eyelid basal cell carcinoma (1); larynx carcinoma (1); uterus carcinoma (1); and thymoma (1).

balance of all of the variables considered. In fact, no statistical difference between the two groups was found with respect to the major characteristics of the patients. The overall protective effect from vomiting and nausea of the two doses of MTC is documented in table 2 and is shown to be similar in the two groups of patients.

Complete protection was achieved in 38.5% of the patients (35.6% in the 60-mg group and 41.5% in the 120-mg group); major protection was obtained in 21.2% (25.5%/16.9%); minor protection was obtained in 20.6% (18.7%/22.6%); and failure of treatment was found in 19.6% (20.2%/19.0%). The overall protective effect of MTC against nausea was also similar in the two groups of patients (table 2), irrespective of the method of evaluation employed. Complete protection (no nausea) was achieved in 52.9% (49.2%/56.6%) of the patients. When intensity of nausea was considered, we found that 27.5% (30.5%/24.5%) of the patients scored their nausea as slight, 15.9% (18.6%/13.2%) scored their nausea as moderate, and 3.7% (1.7%/5.7%) scored their nausea as severe. When the linear visual analog was used, the results were not very different, since 23.1% (25.5%/20.8%) of the patients had the arbitrarily selected levels defined as slight nausea, 14.9% (18.6%/11.3%) as moderate nausea, and 9% (6.7%/11.3%) as severe nausea.

When we analyzed the above results according to the main patient factors which could influence the response to therapy (multifactorial analysis), we found that the sex, age, and dose of cisplatin used were all clinically important (table 3). All of the dependent variables shown in table 3 have significantly higher mean values for the females than for the males. There were vomiting episodes in 81.6% of the females (mean number, 4.29) and in 51.4% of the males (mean number, 2.01). Nausea was present in 63.2% of the females (mean score of nausea, 1.08; mean value of visual analog, 2.91) and in 39.2% of the males (mean score of nausea, 0.51; mean value of visual analog, 1.42, respectively).

Significant age effects were also found but only for the mean numbers of vomiting episodes in the youngest (4.62) and the oldest (1.80) patients, and for the mean values of score of nausea, again in the youngest (1.31) and in the oldest (0.45) patients. Moreover, the dose of cisplatin infused is an important variable. In fact, the mean number of vomiting episodes was significantly higher in the patients who received the highest doses of cisplatin ($\geq$ 100 mg/m^2): 4.94 episodes versus 2.68 and 2.33 episodes observed, respectively, in the patients who had received the intermediate (80–99 mg/m^2) and the low ($<$ 80 mg/m^2) doses of cisplatin. In the same way the

TABLE 2.—Results of antiemetic therapy

	MTC (60 mg)	MTC (120 mg)	*P* value*
No. of emetic episodes			
Average	2.8	2.7	NS
Range	0–14	0–13	
Maximal intensity of nausea			
Score			
Average	0.7	0.7	NS
Range	0–3	0–3	
Linear analog			
Average	1.9	1.9	NS
Range	0–10	0–10	

*P is significance level corresponding to the Mann-Whitney U test. NS = not significant.

TABLE 3.—Analysis of variance by generalized linear models*

Characteristic	Presence of vomiting episodes†	*P* value‡	No. of vomiting episodes†	*P* value‡	Presence of nausea†	*P* value‡	Score of nausea†	*P* value‡	Linear visual analog†	*P* value‡
Sex										
F vs M	2.93	0.005	6.71	0.001	2.38	0.02	2.88	0.01	2.60	0.01
Age										
O vs Y	0.07	NS	-2.59	0.02	-1.51	NS	-2.02	0.05	-1.04	NS
O vs M	1.13	NS	-1.76	NS (0.1)	-1.31	NS	-1.01	NS	-0.21	NS
Dose of cisplatin										
H vs L	1.14	NS	5.14	0.001	2.25	0.05	1.83	NS (0.1)	2.31	0.05
H vs M	0.46	NS	2.21	0.05	1.04	NS	0.98	NS	1.05	NS
Dose of MTC										
h vs l	0.68	NS	0.96	NS	-0.68	NS	-0.28	NS	-0.01	NS
Interaction: cisplatin by MTC doses										
(H vs L) (h vs l)	—	—	-2.57	0.02	—	—	—	—	—	—
(H vs M) (h vs l)	—	—	-2.27	0.05	—	—	—	—	—	—

*t test (with 103 degrees of freedom) and corresponding significance levels for the linearly independent contrast between levels of factors and of interaction. Interaction was introduced only in the model for the No. of vomiting episodes. F and G tests reached significant levels when t test for the contrasts between levels of factors and interaction showed significant differences. For this reason, the table with F and G test values was not shown.

†t test values.

‡NS = not significant.

presence of nausea and its intensity evaluated by the linear visual analog in the patients treated with the highest doses of cisplatin (76.5%; 3.56, respectively) were significantly higher than in those treated with the lowest doses (40.8%; 1.60). No statistical significance was detected between mean values of analyzed dependent variables corresponding to the two MTC levels, but the interaction between cisplatin and MTC doses for the number of vomiting episodes was found to be significant. In fact, if the mean number of vomiting episodes increases with the cisplatin dose, the growth is significantly lower in patients treated with 120 mg of MTC (from 2.45 to 3.57 episodes) than in those treated with 60 mg of MTC (from 2.21 to 5.90). If we analyze, with the same statistical methods, the efficacy of treatment according to the diseases or performance status of the patients, no significant differences were found between the two groups. In particular, the female/male difference was not accounted for by the type of cancer.

The antiemetic treatment was well-tolerated (table 4). Only three patients (2.2%) were forced to interrupt therapy due to the occurrence of side effects: one for an extrapyramidal reaction with 120 mg of MTC and two for severe diarrhea (one with 120 mg and one with 60 mg of MTC). The pattern of side effects was also rather similar between the two treatment groups; the only exception was occurrence of slight sedation which was significantly more frequent in the patients treated with 60 mg of MTC ($P < 0.005$ with respect to Fisher's exact test) and the extrapyramidal reactions which were observed only in two patients treated with 120 mg of MTC.

DISCUSSION AND CONCLUSIONS

High-dose MTC is the treatment of choice for cisplatin-induced nausea and vomiting (4), but the optimal dose of MTC has not yet been established and the most frequently used dosage in published studies is 1 or 2 mg/kg iv repeated for two to six administrations.

The present study is the first randomized double-blind study that attempts to identify the optimal dose of antiemetic treatment by comparing two fixed high doses of MTC. Our patients had not been previously treated with any chemotherapeutic anticancer agent and the evaluation of the antiemetic effect was performed in patients who had been treated only with cisplatin, since the associated cytotoxic drugs were administered after completion of the evaluation period (24 hours). In this way, we have avoided some confounding factors (ie, previous chemotherapy and combination chemotherapy), which have made the results of previous studies difficult to interpret with regard to the efficacy and safety of different MTC dosages used.

TABLE 4.—Toxicity of antiemetic treatment

Toxic effect	No. (%) of patients treated with— MTC, 60 mg ($N = 72$)	MTC, 120 mg ($N = 62$)
Diarrhea*	11 (15.3)	10 (16.1)
Extrapyramidal reaction†	0	2 (3.2)
Slight sedation‡	20 (27.8)	5 (8.1)
Dry mouth	5 (6.9)	4 (6.5)
Nervousness	8 (11.1)	11 (17.7)
Headache	7 (9.7)	7 (11.3)

*> 3 bowel movements in 24 hrs.

†One patient presented akathisia and another trismus and oculogiric crisis.

‡Ref 7.

The overall total and major protective effect (approximately 60% of patients) obtained in our study is similar to that found in previously published reports, irrespective of the MTC dose used. In fact, our trial failed to show any statistically significant difference between the two fixed-dose regimens (60- and 120-mg dose four times within 6 hours) with regard to the number of emetic episodes and intensity of nausea. However, if we consider the patients who received ≥ 100 mg/m^2 of cisplatin and who had significantly more vomiting than those treated with doses < 100 mg/m^2, we find that the higher dose (120 mg) of MTC seems more efficacious than the lower one (60 mg). A possible dose-response relationship between MTC and cisplatin has been suggested in two open uncontrolled studies, and the results of our trial add evidence to these impressions (5,8). Unfortunately, there were very few patients treated with a dose of cisplatin ≥ 100 mg, and we thus believe that this result should be confirmed in a larger group of patients.

In contrast with other investigators (8), however, the escalation of the MTC dose to 120 mg iv for the subsequent cycle of chemotherapy in a subgroup of 18 patients who had failed to achieve a clinically satisfactory result (complete plus major protective effect against vomiting) with the 60-mg MTC regimen failed to improve antiemetic control (results not shown). Whether this was due to other complicating factors arising with subsequent chemotherapy courses, such as diminution of antiemetic efficacy with repeated treatments, or to individual refractoriness is not clear.

MTC serum levels were not measured in our study and this area requires further investigation to define the optimal dose of MTC in cisplatin-treated patients, since it has recently been shown that there may be a threshold blood level of MTC for obtaining an antiemetic effect (15).

Our data evidenced a previously unreported finding that nausea and vomiting are more frequent in women and in those patients < 50 years of age irrespective of antiemetic treatment received.

The incidence of side effects in our series was remarkably low, with no specific pattern for either treatment. Specifically, the overall 1.49% incidence of extrapyramidal reactions seems reassuring with respect to the highly

variable incidence reported in the literature (3%–22%). Figures for diarrhea (15%) are close to the average values reported so far (10%–44%) (5–14). The occurrence of slight sedation was more frequent in the lower dose group of patients and the reasons for this are not clear. This side effect should perhaps not be viewed as undesirable, however. Low MTC toxicity and the fact that antiemetic therapy in cisplatin-treated patients can be concentrated in a 6-hour period make this type of treatment possible on an outpatient basis.

In conclusion, our study suggests that the higher dose of MTC may be necessary for optimal control of nausea and vomiting only in those patients being treated with > 100 mg/m^2 of cisplatin. In this way, the risk of extrapyramidal reactions found more frequently with the highest dose of MTC may be reduced. Moreover, our trial has also shown the need for improvement in current antiemetic therapy, since at least 40% of cancer patients who receive chemotherapeutic regimens including cisplatin are exposed to a disturbing amount of vomiting episodes and nausea.

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Cisplatin Plus 5-FU for the Treatment of Adenocarcinoma of the Colon[1,2]

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Cisplatin plus 5-FU appears to have significant additive activity in various tumors, such as head and neck carcinoma and esophageal cancer. A partial explanation for this may be drug synergism, which has been noted in the L1210 leukemia model. Based on these data, a prospective trial of weekly bolus 5-FU (15 mg/kg) and cisplatin (60 mg/m^2) given every 3 weeks was initiated at Indiana University. Forty-one patients, of whom 38 are fully evaluable for response, were treated with these two drugs. Ten partial and one complete response (complete + partial response rate = 29%) were observed in the 38 evaluable patients. Thirteen additional patients had stable disease for ≥ 3 months. The median durations of remission and survival time were 6 and 10.3 months, respectively. Myelosuppression was unusually severe, with granulocyte counts < 1000/mm^3 in 65% of patients, including four patients with granulocyte count nadirs < 100/mm^3. Three patients developed granulocytopenic fever, with two drug-related deaths (sepsis, hyperosmolar coma). Nearly all patients had nausea and vomiting, but this was not a treatment-limiting toxic effect in any patient. Although this combination suggests a higher response rate than usually seen with bolus iv 5-FU in colon cancer, a trial comparing 5-FU alone or with cisplatin to determine whether true synergy exists is currently underway. [Cancer Treat Rep 69:1359–1363, 1985]

Colorectal adenocarcinoma ranks as the second leading cause of cancer-related death in the United States. Over 130,000 new cases are diagnosed yearly, with nearly one-half of these patients destined to die from disseminated disease (1). With the objective response rate at 15%–20%, 5-FU remains the single most active drug in colonic carcinoma. 5-FU, alone or in combination with other drugs with minimal activity (eg, nitrosoureas, vincristine, streptozocin), has had no obvious impact on overall survival (2).

Little is known regarding the activity of cisplatin in previously untreated patients with colon cancer. In preclinical data, cisplatin is one of the most active drugs in the LoVo colon carcinoma cell line (3). Clinical verification of activity in humans, however, has been poorly delineated. In heavily pretreated patients with metastatic colon cancer, several studies have failed to demonstrate activity for cisplatin (4–7). Using a cisplatin dosage of 50 mg/m^2 every 5 weeks, Kovach et al (4) found no responses in 32 patients (18 previously treated). Similarly, Samel et al (5) reported no responses in 17 evaluable patients with colon cancer using two different dosage schedules of cisplatin. All but one of these patients, however, had previously received chemotherapy (5). In previously untreated patients with adenocarcinoma of the colon, however, cisplatin may have definite activity. Lehane and colleagues (8) treated 30 patients with metastatic or recurrent colon cancer with intra-arterial cisplatin and reported 18 partial and three complete responses. Cisplatin has also been demonstrated by several investigators to have definite single-agent activity for another enteric adenocarcinoma, stomach cancer (9–11). The Southeast Cancer Study Group is currently conducting a trial of cisplatin in previously untreated patients with colon cancer.

The combination of cisplatin plus 5-FU has been associated with high response rates in relatively chemotherapy-refractory diseases such as esophageal (12,13) and squamous cell carcinoma of the head and neck (14). This

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activity may, in part, be explained by the preclinical synergy of these two compounds demonstrated by Schabel and colleagues (15) in the L1210 leukemia model. Whereas neither of these two drugs demonstrates an appreciable difference over control in this model, the combination of cisplatin plus 5-FU resulted in a 60% long-term survival.

Based on this rationale, a phase II trial of cisplatin plus bolus 5-FU was initiated at Indiana University for the treatment of previously untreated metastatic colorectal carcinoma. This report summarizes the results of this study.

MATERIALS AND METHODS

Between October 1982 and September 1983, 41 patients were considered eligible and were entered in this study. All patients had biopsy-proven adenocarcinoma of the colon or rectum with at least one bidimensionally measurable lesion. Malignant hepatomegaly could be used as a measurable lesion if the liver edge was palpable at least 5 cm below the costal margin during quiet respiration. All patients were previously untreated with chemotherapy, and none received radiotherapy within 1 month of entering the study. Further requirements included a serum creatinine of ≤ 2.0 mg/dl, a Karnofsky performance status of $\geq 50\%$, and written informed consent. Characteristics of the patient population are shown in table 1.

Pretreatment studies included a history and physical examination, chest roentgenogram, cbc with platelet count, SMA-12, serum creatinine, BUN, and CEA. Computerized axial tomographic (CT) scan and/or liver spleen scan were performed as indicated for tumor measurement. When CT scans were used as tumor measurement, attempts were made to ensure tumor measurements from comparable levels of prior scans.

Between treatment courses, a cbc with platelet count was obtained weekly. Tumor measurements (by physical exam), as well as serum CEA, BUN, and creatinine, were performed every 3 weeks. Scans necessary for tumor measurement and SMA-12 were repeated every 6 weeks.

Treatment Regimen

Pretreatment hydration consisted of 1 L of 5% dextrose in 0.9 normal saline infused over at least 2 hours prior to cisplatin treatment. Posttreatment hydration was identical to pretreatment hydration. Cisplatin (60 mg/m^2) was administered in 250 ml of 0.9 normal saline and infused over approximately 1 hour every 3 weeks for six courses. 5-FU (15 mg/kg) was administered as a slow iv push weekly for 18 weeks. When the absolute granulocyte count was $< 1500/mm^3$ or the platelet count was $< 90{,}000/mm^3$, treatment was delayed until resolved and was then reinstated with a 25% reduction of 5-FU only. All patients were scheduled to receive six cycles of therapy (18 weeks) unless undue toxicity or progressive disease developed. Patients with progressive disease on therapy were removed from the study but were followed for survival data.

Complete response was defined as the complete disappearance of all objective evidence of disease, including normalization of a previously abnormal serum CEA. Partial response was defined as a $\geq 50\%$ decrease in the sum of the product of perpendicular diameter of measurable lesions. Partial remission was defined as at least a 30% decrease in the sum of the measurements of the liver below each costal margin (at the right and left midclavicular lines) and xiphoid process, in cases where hepatomegaly was the sole parameter.

Survival was measured from the first day of treatment, and response duration was measured from the time of response, according to the method of Kaplan and Meier (16).

Three patients were not considered evaluable for response (one had probable gastric primary, one had had prior chemotherapy, and one was lost to follow-up), but were followed for time to disease progression and death. Two patients experienced early death of 1-2 weeks secondary to sepsis and hyperosmolar nonketotic coma, respectively. These two patients were analyzed as nonresponders. Thus, 41 patients were eligible and 36 patients were considered fully evaluable for this study.

RESULTS

Ten partial and one complete response were observed, for an overall response rate of 27% for eligible patients and 29% for evaluable patients (95% confidence limits were 16%-46% and 13%-39%, respectively). The me-

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	41
Median age in yrs (range): 60.5 (37-74)	
Sex	
Male	26
Female	15
Median Karnofsky performance status (range): 80 (50-100)	
Prior pelvic radiotherapy	7
Metastatic sites (%)	
Liver	22 (54)
Lung	5 (12)
Liver and lung	9 (22)
Pelvic mass	7 (17)
Abdominal mass	5 (12)
Brain	1 (2)
Bone	1 (2)
Skin	1 (2)

dian duration of remission was 6 months (range, 2-9+). Additionally, 13 patients had stable disease for at least 3 months. Eight of the 11 responses occurred in patients with a performance status score of ≥ 80. Responses were noted in both hepatic (six patients) and pulmonary (four) metastases, or both (one), and were associated with a stable or increased performance status in the majority of the symptomatic patients. Responses were documented by chest x-rays (four patients), abdominal CT scan (five), and radionuclide scan (two). The single complete remission was in a man with resolution of liver metastasis on abdominal CT scan following treatment. An additional woman who presented with a bilirubin of > 10 mg/dl and a CEA of 320 with extensive liver metastasis remains in continuous complete remission by abdominal CT scan and liver function tests for 2 years from the onset of chemotherapy. Her CEA failed to normalize (8.6 ng/ml); thus, she was classified as a partial responder. Her performance status rose from approximately 50 to 100. The median survival time of all patients was 47 weeks with the survival curve demonstrated in figure 1.

Thirty-three patients had elevated pretreatment serum CEA levels, including 15 patients with levels > 100 ng/ml, and seven patients with levels > 1000 ng/ml. In three-fourths of these 33 patients, a ≥ 50% decrease in serum CEA was incidentally noted following the first two courses of cisplatin plus 5-FU. Except for documentation of a complete remission, however, a decrease in CEA was not used as an objective criterion for response.

Toxicity was primarily hematologic, with nadir granulocyte counts < 1000/mm^3 in 65% of the patients. Four patients were noted to have granulocyte counts < 100/mm^3. Three episodes of granulocytopenic fever occurred (two documented cases of gram-negative sepsis), one of which was associated with a fatality. Four patients had thrombocytopenia < 100,000/mm^3. Gastrointestinal side effects included nausea and vomiting in nearly all treated patients, but was not severe enough to preclude treatment in any patient. Mild to moderate diarrhea occurred in seven (18%) patients.

Paresthesia of a stocking and glove distribution (four patients) and ataxia (three) were infrequent toxic effects. One patient developed hyperosmolar nonketotic coma and died 10 days following initial treatment. In retrospect, this patient had mild hyperglycemia noted prior to treatment and had received dexamethasone as part of an antiemetic drug regimen. Only three responding or stable patients received fewer than five of their scheduled six courses of therapy.

DISCUSSION

Chemotherapy has had a meager impact on the survival of patients with metastatic colon cancer. Response rates using 5-FU have ranged from 0%-80% depending upon the investigator, patient population, and param-

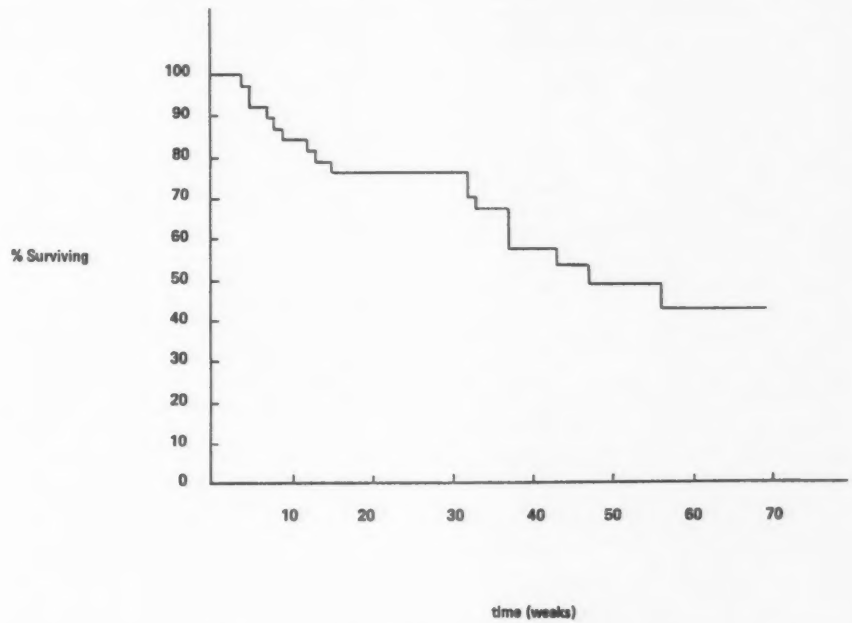

FIGURE 1.—Survival curve for patients treated with cisplatin + 5-FU.

eters used for objective responses (17). Nonetheless, using more stringent criteria, representative response rates for weekly bolus 5-FU average about 15%-20% (2,17).

Preclinical synergy of cisplatin with other chemotherapeutic agents (15) has predicted favorable clinical responses observed in various malignancies. Not only has treatment with cisplatin plus 5-FU appeared to augment the usual clinical responses achieved with single agents in esophageal and head and neck cancers, but similar synergistic combinations of cisplatin plus etoposide have proven successful in other malignancies such as refractory testicular (18) and small cell lung cancers (19-22). In the absence of a randomized trial, one cannot be certain that these drugs are truly synergistic in man. When therapeutic synergism is sought with two or more drugs, however, it is not unusual to expect synergistic toxicity in the host. The marked degree of myelosuppression associated with bolus 5-FU plus cisplatin in this study exceeds the typically minimal myelosuppression seen with either of these drugs utilized in the same dosage as single agents.

The combination of 5-FU plus cisplatin for the treatment of colon cancer has been previously reported by several other investigators. In a phase I clinical trial using various dosages of cisplatin plus 5-FU, Ellerby et al (21) reported responses in four of nine patients with metastatic colonic adenocarcinoma. Cisplatin dosages ranged from 0.625 to 2.5 mg/kg every 4 or more weeks. One of the four responding patients had failed prior treatment with systemic and intra-arterial 5-FU. In a randomized trial, Moertel and colleagues (22) compared very low-dosage cisplatin (20 mg/m^2 on Day 1 only) plus 5-FU (500 mg/m^2/day for 5 consecutive days) repeated every 5 weeks to a combination regimen containing 5-FU plus semustine with or without vincristine. Additionally, all patients were randomized to receive immunotherapy with methanol-extracted residue-BCG or no further therapy. In the cisplatin arms (with or without methanol-extracted residue-BCG), 14 of 56 (25%) treated patients responded, but this response rate was not significantly better than 5-FU plus semustine alone (16%) or with vincristine (23%).

Using continuous-infusion 5-FU (1000 mg/m^2/day for 5 consecutive days) plus cisplatin (100 mg/m^2 on Day 1 only), two groups have recently reported differing response rates for colorectal carcinoma. Shepard et al (23), using cisplatin administered over 4-6 hours, failed to document a single response in 13 previously untreated and seven previously treated patients. In another trial in which cisplatin was administered as continuous infusion over 24 hours, four of ten evaluable patients demonstrated objective responses lasting 3+ to 5+ months (24). Of note is that eight of these patients had progressed during previous 5-FU therapy. Nine additional patients were entered onto the study, but were not considered evaluable.

An alternative explanation for the apparent increased response rate observed in this study is an alteration of metabolism and excretion of 5-FU by cisplatin. The dose-response relationship for 5-FU in colon cancer, however, has been somewhat evasive. Earlier trials with 5-FU suggested that a loading dosage followed by therapy every other day to the point of toxicity was the optimal method of administration (17). The Central Oncology Group subsequently performed a randomized trial comparing this loading regimen, weekly therapy (with or without loading dosages), and oral therapy (25). Although the response rate for the loading regimen was superior (33%) to the other modes of administration (13%-14%), no significant differences in survival or duration of remission were observed. Similarly, the Eastern Cooperative Oncology Group examined three weekly dosage regimens of bolus 5-FU (7.5 mg/kg, 15 mg/kg, and 20 mg/m^2) in patients with metastatic colon cancer, and although respective response rates were 4% (one of 25 patients), 20% (five of 25), and 29% (five of 17), no overall survival advantage was noted for any of the three regimens (26). Further evidence against this possibility of a significant dose-response relationship was demonstrated in a phase I-II trial in which 5-FU was administered with allopurinol by continuous infusion at dosages of 1.25-2.25 g/m^2/day for 5 consecutive days (27). In 37 previously untreated patients, only a 14% response rate was achieved. Thus, despite variation in the response rate, it appears unlikely that a survival advantage might be achieved by altered excretion of 5-FU by cisplatin, but further clinical and pharmacokinetic comparative studies of 5-FU are necessary to clarify this issue.

A dose-response relationship for cisplatin has been demonstrated in testicular and possibly in ovarian and non-small cell lung cancers (28). However, in a well-designed clinical trial examining three different dosage schemes for cisplatin in cervical cancer, no obvious dose regimen relationship could be discerned. If cisplatin is confirmed to have activity in colorectal cancer, the optimal dosage scheme for this drug will remain to be determined.

A final possible explanation for the relatively high response rate seen in this study is the possibility of selection bias, since the majority of the responses occurred in patients with a good performance status. Numerous phase II pilot studies from single institutions using combination chemotherapy have reported apparent increase in response rates over historical data with fluoropyrimidine therapy (2,25). Unfortunately, the vast majority of these regimens have not proven beneficial when subjected to prospective randomized trials. Thus, although the results from cisplatin plus 5-FU appear promising, one should interpret these data with caution. Therefore, we are currently conducting a randomized study comparing cisplatin plus 5-FU to a single-agent 5-FU control arm.

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Phase I Study of Echinomycin Administered on an Intermittent Bolus Schedule[1,2]

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We have conducted a phase I study of the cyclic peptide echinomycin (Quinomycin A) on a schedule of administration of once every 4 weeks. Ten dose levels between 20 and 1800 $\mu g/m^2$ were studied. Acute gastrointestinal toxicity, thrombocytopenia, and transient elevations of serum transaminases occurred at doses of $\geq$ 1000 $\mu g/m^2$. Gastrointestinal toxicity was severe and dose-limiting in several patients at doses of 1800 $\mu g/m^2$. Thrombocytopenia was erratic, but generally increased with drug doses. Platelet count nadirs occurred 5–10 days after administration. Hepatic toxicity was reflected in transient elevations of serum transaminases without hyperbilirubinemia. Three patients experienced apparent anaphylactic reactions to doses of 1500 $\mu g/m^2$. The maximum tolerated single dose of echinomycin was 1800 $\mu g/m^2$. A starting phase II dose of 1500 $\mu g/m^2$ is recommended. [Cancer Treat Rep 69:1365–1368, 1985]

The cyclic peptide echinomycin (Quinomycin A) is a quinoxaline antibiotic originally isolated from *Streptomyces enchinatus* (1). Although the drug is an antibiotic with activity against gram-positive bacteria, recent interest has been focused on its cytotoxic properties. Its mechanism of action may be related to its ability to bind to DNA and inhibit DNA-directed RNA synthesis (2,3). The drug's interaction with DNA takes place through two polar quinoxaline chromophores on opposite sides of the cyclic peptide, both of which can intercalate between DNA base pairs. Thus, echinomycin is a bifunctional intercalating agent, unlike most DNA binders which are monofunctional intercalators. In addition, the compound is specific for double-helical DNA and exhibits base-sequence specificity (4,5).

Echinomycin demonstrated cytotoxic activity against the B16 melanocarcinoma and L1210 leukemia in the tumor panel of the Division of Cancer Treatment, National Cancer Institute (NCI). Because of this activity and its novel mechanism of action, echinomycin was selected for clinical trial. The present phase I trial of echinomycin was undertaken to establish its toxicity when administered as a single 1-hour infusion every 4 weeks, to establish a phase II dose, and to look for initial evidence of therapeutic activity.

MATERIALS AND METHODS

All patients eligible for this study had advanced cancer which was refractory to standard forms of therapy. In addition, they were required to have an expected survival of > 2 months, an Eastern Cooperative Oncology Group performance status of 2 or better, and to have recovered from toxicity of prior treatment. Additional eligibility requirements were: (*a*) adequate bone marrow reserve with a wbc count > 4000/mm^3 and a platelet count > 100,000/mm^3; (*b*) normal renal function, defined as a creatinine < 1.2 mg/dl; and (*c*) stable hepatic function with bilirubin < 1.5 mg/dl and SGOT less than two times normal. Informed consent was obtained from all patients.

Echinomycin was given as a single iv injection once every 4 weeks. The drug was reconstituted in 100 ml of saline and infused over 1 hour. A starting dose of 20 $\mu g/m^2$ was chosen based on preclinical rodent and dog toxicity. On subsequent escalations, patients were en-

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tered at dose levels of 50, 100, 200, 325, 500, 1000, 1250, 1500, and 1800 $\mu g/m^2$. Except for the 50-$\mu g/m^2$ dose, at least three patients were fully evaluated at each dose level prior to entering patients at the next dosage.

Pretreatment studies included cbc with platelet counts, serum chemistry profile (BUN, creatinine, fasting blood sugar, uric acid, SGOT, SGPT, total bilirubin, LDH, calcium, and phosphorus), urinalysis, 24-hour creatinine clearance, chest x-ray, and measurement of all followable disease. To fully evaluate the toxicity of echinomycin during treatment, patients had cbcs twice weekly, and chemistry profiles and creatinine clearance determination weekly. Patients were considered evaluable if they received at least one course of drug with follow-up studies for 4 weeks. Patients remained on study until there was disease progression or toxicity.

RESULTS

Between July 1981 and April 1984, 64 patients were entered in this study. There were 42 males and 22 females, with a median age of 56 years (range, 20–71). Histologic diagnoses of the patients are listed in table 1. Nineteen of these patients had received prior chemotherapy, and 44 had received both chemotherapy and radiation therapy. One patient had received radiotherapy alone. A total of 105 courses were administered (range, one to six) (table 2), and all but three patients were evaluable for toxicity. Only sporadic mild platelet count suppression was seen at doses < 1000 $\mu g/m^2$. At doses ≥ 1000 $\mu g/m^2$, hematologic, hepatic, and gastrointestinal toxic effects emerged.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Male	22
Female	42
Median age in yrs (range): 56 (20–71)	
Median performance status* (range): 1 (0–2)	
Diagnosis	
Colon	29
Melanoma	2
Esophagus	3
Adrenal	1
Breast	3
Renal	1
Ovarian	1
Rectal	2
Pancreas	4
Lung	8
Endometrial	2
Mesothelioma	1
Thyroid	1
Adenocarcinoma of unknown primary	3
Gastric	2
Sarcoma	1

*Eastern Cooperative Oncology Group criteria.

TABLE 2.—Dose escalations of echinomycin

Dose escalations ($\mu g/m^2$)	No. of patients/dose level
20	3
50	1
100	5
200	4
325	5
500	9
1000	8
1250	4
1500	15
1800	10

The hematologic toxicity of echinomycin consisted of thrombocytopenia that occurred about 1 week after the drug was given (table 3). While some decline in platelets occurred in two patients treated at 200 $\mu g/m^2$ and one patient treated at 500 $\mu g/m^2$, the most consistent thrombocytopenia was at doses ≥ 1000 $\mu g/m^2$. Only a few of the platelet count nadirs were $< 100{,}000/mm^3$ (normal range, 150,000–350,000) (table 3). One heavily pretreated patient had a platelet count nadir of $56{,}000/mm^3$ 5 days after receiving a dose of 1000 $\mu g/m^2$. The lowest platelet count nadirs at the 1500- and 1800-$\mu g/m^2$ doses were 79,000 and $113{,}000/mm^3$, respectively. There was no wbc toxicity seen at any dose level.

Evidence of hepatic toxicity occurred in ten patients treated at doses ≥ 1000 $\mu g/m^2$ (table 4). It consisted of transient elevations of SGOT, SGPT, and/or LDH occurring within 10 days of drug administration, and returning to baseline prior to the next course. These patients with elevated liver enzymes were asymptomatic, and there were no cases of associated hyperbilirubinemia. In most cases, the elevations were 1.5–3 times the baseline level. They did not show a cumulative increase with repetition of the dose.

The dose-limiting toxicity of this drug was gastrointestinal. Mild to moderate nausea with onset 4–12 hours after drug administration was seen in five patients treated at 1500 $\mu g/m^2$. The nausea lasted from 12 to 24 hours. One patient had grade IV gastrointestinal toxicity with intractable vomiting that required hospitalization. Six of ten patients treated at 1800 $\mu g/m^2$ experienced significant gastrointestinal toxicity, and one was hospitalized. Patients who had significant nausea during the initial course were given 1–2 mg of droperidol iv prior to further treatment and had partial control of their nausea. Two patients pretreated with droperidol prior to the first course of echinomycin at the 1800-$\mu g/m^2$ dose experienced no gastrointestinal toxicity.

Three patients had apparent anaphylactic reactions to doses of 1500 $\mu g/m^2$, consisting of hypotension,

TABLE 3.—Magnitude of thrombocytopenia at highest doses of ec omycin*

Dose ($\mu g/m^2$)	Patient No.	Baseline platelet count ($\times 10^3$ cells/mm^3)	Nadir platelet count ($\times 10^3$ cells/mm^3)	Day
1500	1	276	92	7
	2	220	156	5
	3	225	162	6
		251	156	7
	4	220	150	6
		215	146	7
	5	181	79	8
		198	150	8
	6	267	162	8
	7	161	83	5
	8	276	92	7
	9	335	162	6
	10	220	156	5
	11-15	†	†	
1800	1	331	240	7
		400	299	12
	2	144	113	7
	3	212	135	7
	4	263	130	10
	5-10	†	†	

* Normal platelet count, 130,000-400,000/mm^3.
†No fall in platelets observed.

flushing, mild bronchospasm, a pruritic erythematous rash, and chills. These reactions occurred early during the infusion and responded to cessation of the infusion and administration of diphenhydramine. One of these reactions occurred during the first course of the drug, while the others were on second courses.

No phlebitis or fever related to drug administration was observed. There were no demonstrable changes in renal function noted, and there were no treatment-related deaths. There were no objective tumor responses seen in the 64 patients entered onto this study.

DISCUSSION

The 20-$\mu g/m^2$ starting dose of echinomycin in this trial was one-thirtieth of the LD10 in mice and was chosen because there was thought to be a steep dose-response toxicity curve in the dog. It subsequently proved to be far below the maximal tolerated dose of echinomycin in humans, and doses < 1000 $\mu g/m^2$ were essentially nontoxic. At doses ≥ 1000 $\mu g/m^2$, gastrointestinal toxicity, platelet depression, and transient elevations of hepatic enzymes became apparent. The most consistent and severe of these toxic effects were the gastrointestinal problems which were of grade 3 or 4 severity in three patients treated at 1800 $\mu g/m^2$. The thrombocytopenia was somewhat sporadic, so that while there was a general increase in its frequency and severity with dose, several patients treated at the highest levels experienced no fall in platelets.

The reasons for the sporadic nature of this toxicity have not been entirely determined, but echinomycin can definitely be expected to produce thrombocytopenia in some patients treated at doses ≥ 1500 $\mu g/m^2$. Finally, the hepatic toxicity observed with this drug was not severe at the doses studied, but the elevations of serum transaminases and LDH were consistent with preclinical studies which demonstrated liver damage by this drug. No renal toxicity of echinomycin was observed in these patients.

The anaphylactoid reactions seen with echinomycin may have been due to the drug or its preservative. The diluent for the drug was polyethoxylated castor oil and ethanol (cremophor), which is known to produce occasional allergic reactions. Allergic reactions have been seen with other drugs such as teniposide, diaziquone, and taxol, which utilize cremophor as diluent (personal communication, D. Schumaker, NCI). Other examples of hypersensitivity to drug vehicles, eg, dimethylacetamide, have been suggested (6). However, several cytotoxic drugs have been associated with hypersensitivity reactions (7), and it is quite possible that a peptide like echinomycin could elicit such a response.

After patient entry and evaluation had been completed at 1800 $\mu g/m^2$, consideration was given to a further dose escalation. The next dose that would have been studied was 2200 $\mu g/m^2$. It was elected not to proceed further, and rather to regard 1800 $\mu g/m^2$ as the maximal tolerated dose when this drug was administered on a single bolus schedule. Part of the reason for this was concern that other investigators conducting trials with echinomycin were encountering some in-

TABLE 4.—Abnormal hepatic transaminase elevations in individual patients*

Echinomycin dose level ($\mu g/m^2$)	SGOT		SGPT	
	Base value	Peak value	Base value	Peak value
20	175	279	57	118
1000	-13	69	53	106
1250	53	121	†	†
1500	46	530	†	†
	118	360	52	155
	19	388	15	397
	†	†	80	327
	†	†	25	101
1800	48	138	53	140
	32	231	†	†

*Normal range of SGOT, 10-42 IU/L; normal range of SGPT, 10-60 IU/L.
†No significant change.

stances of very severe toxicity at doses ≥ 2000 $\mu g/m^2$, and the dose-response for toxicity of this drug would be steep at doses > 1800 $\mu g/m^2$. We have designated 1800 $\mu g/m^2$ as a maximal tolerated dose of echinomycin when the drug is administered on a single bolus schedule, and make the recommendation of a starting dose of 1500 $\mu g/m^2$ for phase II studies when the drug is administered in this fashion. Such phase II studies should contain a provision for cautious dose escalation if individual patient tolerance to echinomycin is satisfactory. Furthermore, while these are the limits for any single dose of echinomycin, the toxicity has shown very prompt resolution and there may not be a need to wait 4 weeks between doses. The results of other schedules of administration, eg, weekly, should be considered in determining the optimum schedule for phase II studies.

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Phase I–II Study of 13-*cis*-Retinoic Acid in Myelodysplastic Syndrome[1,2]

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Eighteen patients with myelodysplastic syndrome received 13-*cis*-retinoic acid (1.0-mg/kg/day starting dose with 0.5-mg/kg increment escalations) in a phase I–II trial. Two partial responses involving the erythroid series were observed in four patients with primary refractory anemia with ring sideroblasts. One of two patients with chronic myelomonocytic leukemia also achieved a partial response. No other responses were found in the remaining patients, which included eight with refractory anemia with excess blasts. In six patients drug toxicity necessitated termination of the trial. Four patients had unexpected drug-induced thrombocytopenia; three of these had low platelet counts before treatment. Two of the six patients had other toxic effects. Further studies are warranted to evaluate the effectiveness of 13-*cis*-retinoic acid in patients with refractory anemia with ring sideroblasts and chronic myelomonocytic leukemia. At moderate doses significant toxic effects, including thrombocytopenia, are not uncommon. [Cancer Treat Rep 69:1369–1374, 1985]

Vitamin A and it derivatives (retinoids) participate in the normal development of epithelial tissues (1). It has recently been demonstrated that retinoids may also influence both normal hematopoiesis and leukemic growth. Retinoids stimulate the growth of early erythroid progenitors in vitro (2) as well as influencing the proliferation and differentiation of in vitro granulopoietic precursors (3,4). Low doses of retinoic acid induced cells from the HL-60 cell line derived from a patient with promyelocytic leukemia to differentiate to morphologically and functionally mature granulocytes (5). Retinoic acid has also been found to inhibit the clonal growth of leukemia cells from five of seven patients with acute myelogenous leukemia (AML) (6).

Myelodysplastic syndrome (MDS) are a group of diseases affecting the hematopoietic stem cell which are characterized by abnormal cellular maturation resulting in ineffective hematopoiesis (7). The frequency and time to evolution into acute leukemia are quite variable (8). At the present time no therapy has resulted in sustained, significantly improved hematopoiesis or postponed the leukemic transformation. We conducted a phase I–II trial of 13-*cis*-retinoic acid (a stereoisomer of retinoic acid) in patients with MDS with the intention of improving the maturation of hematopoietic precursors leading to more effective hematopoiesis. We observed partial responses in two of four patients with refractory anemia with ring sideroblasts (RARS) and in one of two with chronic myelomonocytic leukemia (CMML).

MATERIALS AND METHODS

Patients

Patients with well-established MDS as defined by a modification of the recent French-American-British classification (9) were eligible for this study. Patients with the newly defined category of refractory anemia with excess blasts in transformation were ineligible. For eligibility in the study, the following criteria were required: (*a*) life expectancy ≥ 2 months; (*b*) Karnofsky performance score $> 60\%$; (*c*) no evidence of rapid transformation into acute nonlymphocytic leukemia (ANLL) as defined by a $\geq 50\%$ increase in bone marrow blasts within the preceding 4 weeks if previously $< 15\%$; (*d*) no evidence of an active infectious process, of abnormal baseline liver, renal, or lipid laboratory values, or of abnormal CNS function as evidenced by history or physical

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examination; (*e*) hemoglobin < 10 g/100 ml, and/or absolute granulocyte count < 1000/mm^3, and/or platelet count < 120,000/mm^3; and (*f*) bone marrow aspiration just prior to the initiation of therapy. Informed consent was obtained in accordance with our institutional policies as approved by the Human Subjects Committee.

13-*cis*-Retinoic acid was provided by L. Itri of Hoffmann-La Roche, Inc. Patients were started at an initial dose of 1.0 mg/kg/day orally in two divided doses. The dose was increased in increments of 0.5 mg/kg/day at monthly intervals, until the patient had a maximum dose of 3 mg/kg/day, a satisfactory therapeutic effect, or dose-limiting (moderate level or greater) toxic effects. If three or more moderate toxic effects developed, the dose was reduced by 0.5 mg/kg/day until there were no more than two moderate toxic effects. Our toxicity and side effects scale for 13-*cis*-retinoic acid has been previously published (10). Considering the advanced age of this group of patients and the indolent nature of the hematological disorder, we did not wish to subject them to significant toxic effects for prolonged periods of time. Patients did not receive any other concurrent therapy for their myelodysplastic disorder except that rbc transfusions, if needed, were permitted. Patients were required to receive at least 8 weeks of treatment before being considered a treatment failure.

Patients were seen at least once every 4 weeks while receiving 13-*cis*-retinoic acid. A cbc with differential and platelet count were obtained every 2 weeks for the first 8 weeks and then every 4 weeks. At each clinic visit a history and physical examination were performed, and the following laboratory data were obtained: urinalysis, creatinine, BUN, uric acid, alkaline phosphatase, SGOT, SGPT, bilirubin, cholesterol, triglycerides, high-density lipoproteins, total protein, albumin, calcium, phosphorus, lactic dehydrogenase, prothrombin time, and electrolytes.

Response Criteria

A complete response (CR) was defined as return of the peripheral blood cell counts to normal and a bone marrow aspiration revealing normal erythroid, granulocytic, and megakaryocytic maturation accompanied by a lack of ring sideroblasts for ⩾ 1 month. A partial response (PR) was defined by any one or more of the following for ⩾ 1 month: a ⩾ 50% decrease from baseline in rbc transfusion requirements, increase in hemoglobin level of > 2 g/100 ml without rbc support, ⩾ 50% increase from baseline in absolute granulocyte count, ⩾ 50% increase from baseline in platelet count, or ⩾ 50% decrease from baseline in bone marrow myeloblasts if initially they were > 10%. Stable disease was defined as no significant change in rbc transfusion requirements, peripheral blood cell counts, bone marrow abnormalities, or symptoms for ⩾ 1 month.

RESULTS

Eighteen patients were entered in this study. Their type of MDS and onstudy hemoglobin and wbc and platelet counts are listed in table 1. Patient 2 had received chemotherapy for advanced Hodgkin's disease and his RARS was felt to be secondary to chemotherapy. Patient 3 with RARS had received multiple courses of therapy with ^{131}I for thyroid carcinoma. Patient 5 with refractory anemia with excess blasts (RAEB) had received 6 months of therapy with busulfan for polycythemia vera 17 years prior to entering this trial. None of the other 15 patients had received any prior cytotoxic therapy.

There were no CRs. Two PRs in the group of four patients with primary RARS were observed. Therapeutic effect appeared only in the erythroid series. One patient (No. 12) had an increase in his hemoglobin level for 4 months. Three months after treatment was discontinued a progressive decrease in hemoglobin was noted, necessitating subsequent rbc support. In another patient (No. 1), the rbc transfusion requirement fell by slightly > 50% for 5 months. One of two patients (No. 17) with CMML achieved PR. He had an increase in hemoglobin for 2 months and an increase in granulocytes for 3 months, without any reduction in peripheral blood monocytosis. The other patient with CMML (No. 18) who is still in the study experienced a > 50% reduction in his peripheral blood monocytosis after 2 weeks of treatment, without any improvement in severe thrombocytopenia. None of the eight patients with RAEB met our response criteria; however, three did not have an adequate therapeutic trial because of toxicity. In one patient with RAEB and thrombocytopenia (Patient 10), a transient decrease in bone marrow myeloblasts was observed without an accompanying increase in platelet count.

The toxic effects of the 13-*cis*-retinoic acid in this trial are presented in table 2. The only unexpected toxic effect was drug-induced thrombocytopenia which was observed in four patients, three of whom had low platelet counts prior to starting treatment. In all four patients there was a > 50% decrease in pretreatment platelet counts which improved after cessation of the 13-*cis*-retinoic acid (table 3). One of these patients was a partial responder (Patient 1) and this toxic effect was only noted after 5 months of treatment (4 months at a lower dose). In another patient (No. 15), a further decrease in platelet count was observed 1 week after the discontinuation of 13-*cis*-retinoic acid, necessitating the administration of steroids. In two other patients therapy had to be discontinued because of toxicity; one because of severe emotional lability and headache (Patient 7) and another because of severe epistaxis and fatigue (Patient 14). All of these side effects subsequently resolved entirely. A total of six patients had their therapy stopped because of drug toxicity. Details of severe grade toxic effects are presented in table 4. Because of toxicity, no patient could be escalated to the

TABLE 1.—Patient

Patient No.	Type of MDS	Hemoglobin (g/100 ml)	Wbc count ($\times 10^3$ cells/mm^3)	Platelet count ($\times 10^3$ cells/mm^3)	Durati of treatm (wks
1	RARS	9.5	4.1	156	23
2	RARS secondary to therapy	8.2	2.7	176	15
3	RARS	9.5	2.4	65	10
4	RA	10.5	4.7	27	33
5	RAEB	13.4	7.6	60	43
6	RAEB	8.7	1.8	18	4.5
7	RAEB	9.9	14.3	14	2
8	RA	9.8	2.0	38	16
9	RAEB	6.4	0.9	16	8.5
10	RAEB	13.1	14.8	78	11
11	RAEB	9.3	4.2	21	4.5
12	RARS	7.7	5.8	339	30
13	RARS	7.0	5.6	112	22
14	RA	10.5	5.2	100	3
15	RAEB	11.4	3.8	75	2

.—Patient profile and response data

Duration of treatment (wks)	Highest dose achieved (mg/kg)	Drug toxicity necessitating termination of trial	Response*	Comments
23	2.0	+	PR	50% decrease in rbc transfusions for 5 mos
15	2.0	-	NR	History of chemotherapy for Hodgkin's disease; overt ANLL diagnosed 2 mos later
10	1.0	+	MR	History of treatment with multiple courses of ^{131}I
33	1.5	-	SD	Less gingival bleeding and epistaxis but no increase in platelet count; died from sepsis
43	1.0	-	SD	History of treatment with busulfan for polycythemia vera 17 yrs ago
4.5	1.0	+	NE	Evolved into ANNL and died
2	1.0	+	NE	
16	2.0	-	SD	
8.5	1.5	-	NR	Treatment discontinued because of development of disseminated coccidiomycosis
11	1.5	-	SD	Decrease in myeloblasts from 7% to 2% × 4 wks without increase in platelet count
4.5	1.5	-	NR	> 30% myeloblasts in bone marrow at conclusion of treatment
30	2.0	-	PR	Increase in hemoglobin for 4 mos
22	2.0	-	SD	Died from unrelated causes
3	1.0	+	NE	
2	1.0	+	NE	Further decrease in platelet count 1 wk after discontinuing treatment

Continued

TABLE 1.—Patient profile and response data (Continued)

Patient No.	Type of MDS	Hemoglobin (g/100 ml)	Wbc count ($\times 10^3$ cells/mm^3)	Platelet count ($\times 10^5$ cells/mm^3)	Duration of treatment (wks)	Highest dose achieved (mg/kg)	Drug toxicity necessitating termination of trial	Response*	Comments
16	RAEB	10.1	2.2	76	12	1.0	-	SD	
17	CMML	9.5	6.2	161	14	1.0	-	PR	Increase in hemoglobin for 2 mos; increase in granulocyte count for 3 mos without change in peripheral blood monocytosis
18	CMML	11.1	23.0	10	9+(still on study)†	1.0		SD	> 50% decrease in peripheral blood monocytosis without platelet count increase

*NR = no response; MR = minor response; SD = stable disease; and NE = not evaluable.
†Went off study after 10 wks of treatment because of epistaxis. He was considered to have stable disease.

maximum 3-mg/kg/day dose level. Four patients (three with RAEB) were not evaluable for response because of the short duration of treatment (2, 2, 3, and 4.5 weeks) due to drug toxicity. At the present time, a total of three patients have evolved into ANLL as defined by > 30% myeloblasts in the bone marrow (Patients 2, 6, and 11), and five have died (Patients 2, 4, 6, 11, and 13).

DISCUSSION

In this trial we observed limited efficacy and more toxicity than expected at this dose level in patients with MDS. The three responses were all partial and were seen in patients with primary RARS and CMML. In the former, improvement was noted only in the erythroid series. Both patients with CMML derived some therapeutic effect. One achieved PR with improvement in hemoglobin and granulocyte count. The second patient, still receiving treatment, obtained a > 50% decrease in peripheral monocytosis after 2 weeks of treatment. None of the eight patients with RAEB responded, although three did not have an adequate therapeutic trial because of drug toxicity.

Equally important, we observed significant toxicity in this trial. The drug-induced thrombocytopenia was not anticipated because it was not observed in 240 patients with nonhematologic neoplasms we have treated over the past several years with 13-*cis*-retinoic acid in comparable doses in a series of phase I and II studies (11). However, these patients all had normal platelet counts in contrast to the pretreatment thrombocytopenia in three of four patients in this present study who developed drug-induced thrombocytopenia. We also observed other side effects graded as severe including dry skin, cheilitis, conjunctivitis, headache, fatigue, anorexia, epistaxis, emotional lability, and serum triglyceride elevation.

Gold et al (12) recently reported the results of a phase I clinical trial of oral 13-*cis*-retinoic acid in patients with MDS. They observed five responses in 15 evaluable patients. All were PR and were observed at doses ranging from 20 to 125 mg/m^2/day. Three of ten patients with RAEB and two of four with CMML responded. In this study there were no patients with RARS and only one evaluable patient with RA. In general, the toxicity was acceptable, with hepatotoxicity being the dose-limiting toxic effect. All toxic effects were completely reversible and these investigators considered a dose of 100 mg/m^2/day to be safe.

Our results differ from Gold et al (12) in two respects. We did not observe any responses in our eight patients with RAEB. This lack of efficacy remains unexplained but cannot be attributed to our dose level, since these investigators reported responses in patients with RAEB at doses of only 20 and 45 mg/m^2/day. However, we used a split-dose schedule, while the patients in Gold's study received a single daily dose.

TABLE 2.—Toxic effects of 13-*cis*-retinoic acid in patients with MDS*

Type	Degree		
	Mild	Moderate	Severe
Dry skin	8	3	1
Cheilitis	7	3	1
Alopecia	3		
Pruritus	3		
Conjunctivitis	3		1
Epistaxis	4	4	1
Headache		2	2
Fatigue	3	2	1
Anorexia	1		1
Emotional lability			1
Thrombocytopenia (drug-induced)			4
Elevation in			
Serum alkaline phosphatase		1	
Serum LDH		1	
Serum triglyceride			3†

* Values = No. of patients.

†In 1 patient the baseline value was slightly elevated and in the other 2 the values were approximately twice normal.

In addition, Gold et al (12) did not observe any drug-induced thrombocytopenia, even in patients who had significant thrombocytopenia before treatment. This was the most frequent severe toxic effect in our study, leading to cessation of treatment in four patients. Although we escalated the dose in each patient while Gold did not, this cannot explain the frequent occurrence of drug-induced thrombocytopenia, since this occurred at the starting dose of 1.0 mg/kg/day in three of the four patients. Furthermore, we have not observed this toxicity in any of 18 patients with mycosis fungoides treated with a twice-a-day schedule (11).

The results of in vitro studies demonstrating beneficial effects on erythropoiesis, granulopoiesis, and leukemic proliferation and differentiation provide a sound rational basis for therapeutic trials of retinoids in acute leukemia and MDS (2–6). The results of our trial suggest that 13-*cis*-retinoic acid may have activity in patients with RARS and CMML but as a single agent at the doses we employed it was not effective in RAEB. One should also be aware that 13-*cis*-retinoic acid has the potential for accelerating leukemic growth in vitro. We observed an enhancement of leukemic proliferation in response to 13-*cis*-retinoic acid in cultures from four of 15 patients with

TABLE 3.—13-*cis*-Retinoic acid-induced thrombocytopenia

Patient No.	Dose (mg/kg)	Duration at dose (wks)	Platelet count ($\times 10^3$ cells/mm^3)			Comments
			Pretreatment	When treatment discontinued	Posttreatment	
1	2.0	6	156	59	100	Had been treated for 4 mos at lower doses before this toxic effect was noted
2	1.0	4	65	32	61	
6	1.0	4.5	18	3	20	
15	1.0	2	75	39	89	1 wk after treatment discontinued, platelet count decreased to 24×10^3 cells/mm^3 and steroids were begun

TABLE 4.—Severe grade toxic effects (excluding thrombocytopenia)

Type	Patient No.	Dose (mg/kg/day)	Duration at dose (wks)
Dry skin	7	1.0	2
Cheilitis	8	2.0	4
Conjunctivitis	11	1.5	1
Epistaxis	14	1.0	3
Headache	4	1.5	4
	7	1.0	2
Fatigue	14	1.0	3
Anorexia	8	2.0	4
Emotional lability	7	1.0	2
Elevation in serum triglyceride	2	2.0	4
	8	2.0	4
	17	1.0	4

MDS (Durie BGM, et al, manuscript in preparation). This same observation has recently been reported by Lawrence et al (13) in cultures from patients with ANLL. Whether this in vitro phenomenon may also occur in vivo is unknown. The potential for accelerating leukemic growth with 13-*cis*-retinoic acid in clinical practice remains to be defined, particularly since proliferation is initially required in most biological systems for differentiation and maturation to occur, although it has been convincingly demonstrated in the HL60 cell line that retinoic acid induces differentiation without requiring proliferation (5).

The improvement in erythropoiesis we observed in two patients with RARS warrants further trials of retinoids in patients with RA and RARS, since depressed erythropoiesis is the most significant problem in this group of patients. The therapeutic effects in CMML observed by Gold et al (12) and us requires confirmation with larger numbers of patients. This agent may have a role in the management of this disorder associated with a poor prognosis and poor response to conventional cytotoxic drugs other than perhaps low doses of cytarabine (14). Retinoids combined with other agents capable of promoting differentiation of leukemic cells should be explored.

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Randomized Phase II Clinical Trials[1]

Richard Simon,* Robert E. Wittes, and Susan S. Ellenberg[2]

The sources of variability influencing the results of phase II trials are reviewed. Randomized designs for phase II testing are presented and evaluated. Phase II designs with "standard therapy" control groups are not found to be broadly useful. Designs which randomize among new agents or schedules appear to be of value both scientifically and logistically where patient accrual is adequate. The rationale and advantages of this design are described. The concept of ranking and selection of new agents and schedules is presented as an alternative to testing the null hypothesis of therapeutic equivalence. Sample size calculations demonstrate potential advantages of this approach in appropriate situations. The conduct of pilot or "phase II" studies of combinations is also discussed and randomized designs with early stopping rules are proposed. [Cancer Treat Rep 69:1375–1381, 1985]

Better drugs are needed for the treatment of most human malignancies. Consequently, it is important that the clinical evaluation of new agents be carefully performed. Phase I and II studies present special difficulties, because they involve use of agents whose spectrum of toxicity and likelihood of benefit are poorly defined. It is the purpose of this paper to review problems with phase II studies and to explore the potential role of randomization in such trials.

Objectives of Phase II Trials

There are three basic objectives in treating patients on phase II studies (table 1). The first is to benefit the patients. This must be a primary objective of any therapeutic intervention. The second objective is to screen the experimental agent for antitumor activity in a given type of cancer. Agents which are found to have substantial antitumor activity and an appropriate spectrum of toxicity are generally incorporated into combinations to be evaluated for patient benefit in controlled phase III trials. The third objective of the phase II trial is to extend our knowledge of the toxicology and pharmacology of the agent. It is useful to clearly distinguish Objective 1 from Objective 2. Response rate is an appropriate endpoint for evaluating Objective 2. We generally cannot adequately evaluate the extent to which Objective 1 is achieved in phase II trials. Response rate is only meaningful to the patient if causing tumor shrinkage means extending survival or improving quality of life. This may or may not be the case. Because an untreated control group is generally not available, one cannot properly evaluate whether the new agent influences survival. Comparing survivals of responders to survivals of nonresponders is not a valid way of demonstrating that there has been an impact of treatment on survival. Such comparisons are biased by the fact that responders must live long enough for a response to be documented. Also, responders may have more favorable prognostic factors than nonresponders, leading to a difference in survival regardless of treatment (1–3). It is even possible that treatment may shorten the survival of nonresponders rather than lengthen that of responders. Figure 1 shows an example in which non-small cell lung cancer responders survive longer than nonresponders, but the composite survival of the entire group is no different from an untreated historical control (4).

TABLE 1.—Objectives of phase II trials

1. Benefit the patients
2. Screen drug for antitumor activity
3. Extend knowledge of toxicology and pharmacology of drug

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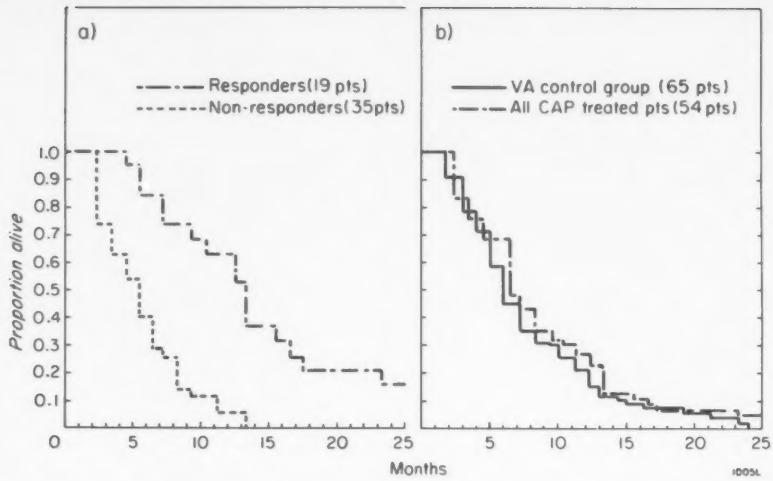

FIGURE 1.—(a) = survival curves (ref 4) for 19 "responding" and 25 "nonresponding" patients with non-small cell lung cancer treated with cyclophosphamide, doxorubicin, and cisplatin; (b) = survival curve derived from the curves in (a) for the whole population of treated patients compared with a historical control group of 65 untreated patients.

Reliability of Phase II Trials

There is frequently great variability in the response rates reported from different phase II studies of the same agent. Some major factors that contribute to this variability are listed in table 2. The first factor is patient selection. Response rates generally decrease as the extent of prior therapy increases. Patients who have failed several prior regimens are more likely to have tumors composed of large numbers of resistant cells, and such patients are also less likely to be able to tolerate full doses of the investigational drug. Whereas it may be appropriate to screen for drugs cytotoxic to tumor cells that are resistant to standard agents, such screening generally cannot be effectively performed in patients with poor bone marrow and other organ system reserves. The steep dose-response relations observed in experimental tumors for some cytotoxic agents suggest that adequate phase II evaluations should be performed at maximally tolerated doses. Probably the most frequent problem with phase II studies is that the patients selected are so debilitated by disease and prior therapy that an adequate evaluation of antitumor activity is impossible. Such patients are less likely to benefit from the drug, and the trial itself will not contribute meaningfully to evaluation of the drug. Debilitated patients are more likely to die or withdraw early in the course of treatment. Some investigators consider the response of such patients inevaluable, and the variable number of such patients contributes to variability in reported response rates.

TABLE 2.—Sources of variability in results of phase II trials

1. Patient selection
2. Response criteria
3. Inter-observer variability in response assessment
4. Dosage modification and protocol compliance
5. Reporting procedures
6. Sample size

A second important factor is variability in response criteria among institutions and groups (5). Some reports of phase II trials do not describe or reference the exact criteria used in sufficient detail to adequately interpret the results. A third factor is subjectivity in assessment of response. There have been few formal evaluations of the impact of measurement error on reported rate of tumor response (6–9). In a recent study, Warr et al (9) compared measurements of several physicians on real or simulated malignant lesions, and found that some commonly used criteria of response are subject to large errors. For this reason, they have recommended requiring more extensive and longer lasting evidence of tumor reduction as a basis for the definition of partial response. A fourth source of variability in results includes differences in dosage modification guidelines and differences in protocol compliance. These features reflect differences in aggressiveness of treatment and carefulness in performing clinical trials. They may also reflect differences in patient selection, as protocol compliance may be more difficult in a population of heavily pretreated patients. The variations in policies for handling exclusions in the calculation of response rates and the small sample sizes of

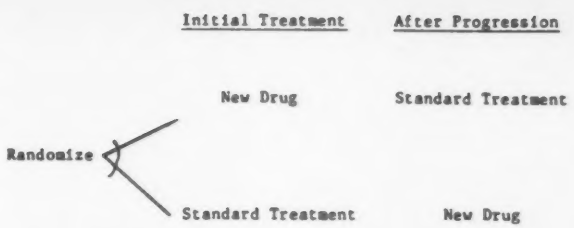

FIGURE 2.—Randomized phase II design of new agent vs positive control treatment.

phase II trials also influence the variability of results (10). Certainly, the outcome of treatment for all patients entered in a trial should be reported regardless of whether the investigator considers some of the tumor responses to be unevaluable.

In the following sections, we shall describe randomized designs for phase II studies and attempt to evaluate whether the problems resulting from the sources of variability listed in table 2 are reduced by such designs.

Randomized Phase II Trials With Control Arms

One type of phase II design which has been employed involves randomization between an investigational agent and an active standard treatment (fig 2). Crossover after progression of disease is shown in the figure. The major objective of the randomization is to help in the interpretation of a poor response rate to the investigational agent. If the new agent has an observed response rate of, say, 25%, then the drug would be identified as having antitumor activity regardless of the magnitude of the response rate to the control treatment. Suppose, however, that the new agent has a low observed response rate (eg, $< 10\%$). In this case, one's conclusion about activity would depend on the response rate observed in the control group. If that response rate is sufficiently large and if the sample size is adequate, then we would conclude that the new agent is inactive for this type of tumor. If the response rates to the new agent and control are both poor, however, then the trial is indeterminate due to inappropriate patient selection. The purpose of randomization here is not to determine whether the new agent is better or worse than the active control.

The design does not seem to offer a broadly useful approach to phase II testing. It is potentially useful in circumstances where an adequate response rate on the active control is not assured, but the identification of an "active control" treatment may be difficult. With the usual phase II sample sizes, it may also be difficult to reliably determine whether the patients are sufficiently responsive to the control treatment for the test of the investigational agent to be meaningful. The expected attrition prior to crossover usually precludes obtaining substantial secondary response rate or cross-resistance information. This design may be useful in some studies involving patients with responsive tumor types who have received several previous treatments. Generally, however, a better approach would be to limit eligibility criteria to patients with less prior therapy instead of employing this design.

Randomized Phase II Trials Without Control Arms

The second type of randomized design is shown in figure 3. Two or more treatment arms are possible with this design and the arms are all experimental agents. When such a design is mentioned, investigators are sometimes puzzled at the rationale for conducting a large randomized comparison of two agents which may have no activity in the disease. Such a proposal would indeed be inappropriate, but the fact that randomization is employed does not mean that phase III-type sample sizes are to be employed nor does it mean that comparison, in the usual sense, is the objective. The design can be performed with sample sizes, and early stopping rules, conventionally used for nonrandomized phase II studies.

There are several advantages to the design shown in figure 3. First, randomization helps ensure that patients are centrally registered before treatment starts (11). Such registration is essential for checking patient eligibility, terminating accrual when the target sample size is reached, and establishing a reliable centralized record of treated patients in order to ensure that all such patients are reported upon. Establishment of a reliable mechanism to ensure patient registration prior to treatment is of fundamental importance for all clinical trials. Randomization facilitates this task, since the protocol treatment is unknown until the patient is registered and the randomization performed.

Other advantages of the randomized design shown in figure 3 compared to independent phase II studies are that differences in results obtained for the two agents will more likely represent real differences in toxicity or antitumor effects rather than differences in patient selection, response evaluation, or other factors listed in table 2. With separate studies, the institutions and participating investigators may differ. Even if the eligibility criteria are identical, the protocol priorities may differ.

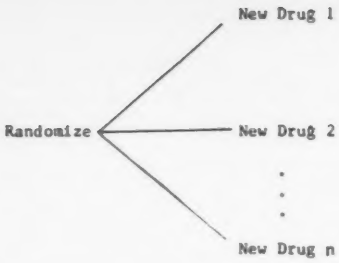

FIGURE 3.—Randomized phase II design of 2 or more new agents.

Thus, patients who are more debilitated and less likely to respond may be entered on one protocol. Rates of patient inevaluability also differ substantially among institutions, and the randomized design tends to distribute inevaluable patients evenly among the treatments. Because partial response assessment is somewhat subjective and of limited reliability, there is an advantage to the randomized phase II study in that uniform second-party review of response for both treatments is more readily recognized as being appropriate.

One may ask why comparability of prognostic makeup or response assessment matters if we are not attempting to compare agents. The answer is that a limited form of comparison is often desired. The objective of most phase II studies involves more than just determining whether the agent has any antitumor activity. It also includes estimating the degree of antitumor activity, the extent of tumor shrinkage, the proportion who respond, and the durability of responses. Although conventional sample sizes limit the precision with which degree of activity can be estimated, the estimation is important. Such estimates are regularly used to rank available agents in selecting therapy for patients and in developing plans for introducing the investigational agent with the most promising phase II results into front-line combinations. Even though the sample sizes in randomized phase II studies may not be sufficient to test hypotheses of equality of effect, the inherent comparability of results assures that drugs can be reliably ranked when large differences are obtained. The comparative aspect of phase II trials is even more obvious in cases involving analogs, different schedules of the same agent, different preparations of the same drug, or different agonists and antagonists of a physiologic substance. Some analog studies, in fact, should probably be designed as large-scale phase III trials employing early stopping rules to handle the contingency that an analog could be totally ineffective.

Sample Sizes for Selecting Among Drugs or Schedules

In the previous section, we have tried to indicate the advantages of the design shown in figure 3 for purposes of ranking and selecting agents and schedules for further study. Whereas traditional procedures for determining sample sizes for nonrandomized phase II trials remain generally applicable (12), there are also circumstances where the direct methods of statistical ranking and selection theory are useful (13). Since these methods have not been described in the oncology literature or applied to cancer clinical trials, they will be presented briefly here. With the statistical selection theory criterion, one always selects for further study the treatment with the greatest response rate (or best value of the other primary endpoint used, eg, lowest incidence of toxicity), regardless how small or "nonsignificant" its advantage over the other treatments seems to be. The sample size is established to ensure that if a treatment is superior to all the others by an amount D, then it will be selected with high probability P. If there are two groups of treatments and the best ones are of equal efficacy with a response rate D greater than those in the other group, then the selection therapy approach ensures that one of the better treatments will be selected with a high probability P. Table 3 shows the required number of patients per treatment with $P = 0.90$ and D = 15%. For example, suppose that three schedules of administration are to be studied and the expected baseline response rate is 20%. With 44 patients per schedule, we have probability 0.90 of selecting the schedule that has a true response rate of 20% + 15% = 35%. This compares favorably with a sample size of 91 per group for a phase III comparison of two treatments with power 0.90 for detecting differences of 20% with a two-sided 5% significance level (14). Using the selection theory approach, a treatment will still be selected even if the schedules are equivalent or the differences are < 15%, but the probability of correct selection (if there are small true differences) will not be as great as 90%. Conventional statistical designs used in clinical research require much larger numbers of patients, because they select a treatment as superior only when the data are incompatible with the hypothesis that the treatments are equivalent. Selection theory designs always select a treatment as superior, even if they are actually equivalent. With these designs, the probability of selecting a treatment which is actually inferior by amount D (eg, 15%) is limited to be 1-P; however, the concepts of significance level and power do not have direct analogs in selection theory. More complex selection theory designs involving sequential analysis have also been studied (13).

Although there is substantial statistical literature on methods for determining sample sizes for phase II trials, the issue of patient heterogeneity is rarely discussed. For trials in which both previously treated and non-previously treated patients with the same type of cancer are eligible, the results should generally be analyzed separately for the two groups, since response rates are often so different. For such a study, the number of non-previously

TABLE 3.—No. of patients/treatment for selection designs*

Smallest response rate (%)	No. of treatments		
	2	3	4
10	21	31	37
20	29	44	52
30	35	52	62
40	37	55	67
50	36	54	65
60	32	49	59
70	26	39	47
80	16	24	29

* Probability of correctly selecting best treatment is 0.90 when it is superior by absolute difference of 15% in response rate.

treated patients should be sufficient for separate analysis, and previously treated patients should probably not be entered on study until results for the former group indicate that the agent has sufficient antitumor activity. There is little potential benefit in administering an agent to previously treated patients which is inactive in non-previously treated patients.

Phase II Studies of Combinations

Though this paper is primarily oriented to evaluating single agents, there are also numerous small "phase II" studies of combinations. For some diseases, there are many active combinations, but it is difficult to know which components contribute to the activity or how to build more effective combinations with tolerable toxicity. Combination studies are clearly oriented towards determining level of activity rather than just presence of activity. Consequently, the problems listed in table 2 are even more severe for pilot studies of combinations. For two-drug combinations, a useful solution, at least for some diseases, would be to conduct randomized pilot phase III trials in which the combination is compared to one of the component single agents as indicated in figure 4. The sample size would be planned as for a usual phase III trial, but accrual would be terminated early if preliminary results indicate that the combination is unlikely to produce a clinically meaningful improvement over the single agent. There are a variety of sequential designs available which effectively accomplish this (15,16). The same approach can be used to assess the contribution of a drug to a multidrug combination (eg, ABC vs AB). Sequential designs are less practical, however, if the endpoint is survival and takes a long time to be observed. Chalmers (17) has emphasized the importance of such randomized pilot studies for avoiding over interpretation of initial results on highly selected patients. Use of this approach, perhaps with a 2 to 1 randomization weighted in favor of the combination, could facilitate the development of effective combinations and reduce the number of patients treated on studies whose results are ambiguous. The 2 to 1 weighted randomization may be desirable in some trials, as it permits accumulation of greater experience using the new combination with little loss in power compared to an equal randomization (eg, about 13% more patients are required) (18).

DISCUSSION

In this paper, we have attempted to outline problems in phase II evaluations of chemotherapeutic agents and to explore whether ramdomization is of value in solving these problems.

The randomized design shown in figure 2 may be useful in some circumstances where the appropriateness of the patient population for phase II trials is in question. Although this is often the case, a better solution to the problem would be to utilize patients with less prior therapy. For unresponsive solid tumors, it would also be difficult to identify an active control treatment for use in this design.

The design shown in figure 3 is of much broader usefulness. For settings where patient accrual rate is sufficient, it contributes in several ways to the interpretability of phase II results. The design also facilitates the logistics of conducting phase II trials of common tumors and is used extensively by some cooperative groups. When accrual is adequate, the only substantial disadvantages of this design seem to be the necessity of discussing randomization as part of the informed consent process, and the potential for misinterpreting the results as if they were from a large comparative phase III trial (19). For purposes of ranking and selecting drugs and schedules for further study, however, the randomized design of figure 3 is of value when several drugs or schedules are available. The selection theory approach to planning sample size for such studies is also worthy of consideration.

Phase II trials are problematic in diseases such as prostatic, pancreatic, or brain cancers because of the difficulties in evaluating response. In such circumstances, one may be forced to use survival as the primary endpoint. A new agent could be directly evaluated in a phase III comparison to either a standard treatment or a palliative treatment control; however, an alternative approach is to design a trial to select among several new agents. Surviv-

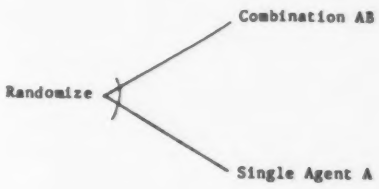

FIGURE 4.—Randomized phase II/III design for evaluating new combinations.

al would be the endpoint, but the trial would require fewer patients per new agent than a phase III trial. The selection trial would then be followed by a phase III comparison of the selected treatment to the standard or palliative control.

The design shown in figure 4 seems particularly useful for studies of new drug combinations (eg, AB or ABC) in which a single agent (A) or combination (AB) serves as control. Although small pilot studies are required for ensuring tolerability of a new regimen, such designs with appropriate early stopping rules provide more reliable screens for improved combination therapy.

APPENDIX

We present here the formula used for calculation of the numbers in table 3. The treatment selected is that with the largest observed response rate. For selecting among K treatments when the difference in true response rates of the best and next best treatment is D, the probability of correct selection is smallest when there is a single best treatment and the other K-1 treatments are of equal but lower efficacy. If we stipulate that the response rate of the worst treatment equals a specified value p, then the probability that the best treatment produces the highest observed response rate is:

$$\sum_{i=0}^{n} f(i)\,[\,1 - B(i\,;\,p+D\,,\,n)\,] \qquad [1]$$

where B(.; p + D, n) is the cumulative distribution function for a binomial distribution with success parameter p + D and n patients and f(i) is the probability that the maximum number of successes observed among the K-1 inferior treatment is i. Thus,

$$f(i) = [\,B(i\,;\,p\,,\,n)\,]^{K-1} - [\,B(i-1\,;\,p\,,\,n)\,]^{K-1}$$

If there is a tie among the treatments for the largest observed response rate, we shall assume that one of the tied treatments is randomly selected. Hence, in calculating the probability of correct selection, we add to expression [1] the probability that the best treatment was selected after being tied with 1 or more of the other treatments for the greatest observed response rate. This probability is:

$$\sum_{i=0}^{n} b(i\,;\,p+D\,,\,n) \sum_{j=1}^{K-1} g(i\,,\,j)\,/\,(j+1) \qquad [2]$$

where
$g(i,j) = \mathrm{COMB}(K-1,j)\,[b(i;\,p,\,n)]^{j}\,[B(i-1;\,p,\,n)]^{K-1-j}$, b denotes the binomial probability mass function and COMB (K-1,j) denotes the number of combinations of K-1 objects takes j at a time. The quantity g(i,j) represents the probability that exactly j of the inferior treatments are tied for the largest number of observed responses among the K-1 inferior treatments, and this number of responses is i. The factor 1/(j+1) in expression [2] is the probability that the tie among the best treatment and the j inferior treatments is randomly broken by selecting the best treatment.

The probability of correct selection is the sum of expressions [1] and [2]. For specified values of p, D, and K, the value of n, number of patients per treatment, is determined to provide a probability of correct selection equal to that desired (*P*).

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Pharmacokinetics of Diaziquone After Three Different Dosage Regimens[1,2]

Thorir D. Bjornsson,* S. Clifford Schold, Henry S. Friedman, Dorothy Schneider, and John M. Falletta[3,4]

Diaziquone (AZQ) is a benzoquinone derivative exhibiting significant effects against CNS tumors. This study investigates the pharmacokinetics of AZQ for three different durations of continuous iv infusions, over 20 mins, 4 hrs, and 6 hrs, in nine patients with CNS malignancies. Total AZQ doses varied from 10 to 20 mg. The average elimination half-life was 37.2 ± 13.9 mins, the total clearance was 515 ± 280 ml/min or 11.3 ± 5.9 ml/min/kg, and the apparent volume of distribution by area was 26.5 ± 14.7 L or 0.62 ± 0.50 L/kg. Peak concentrations after the 20-min infusions (three patients) varied from 0.58 to 1.20 μg/ml, whereas concentrations during the 4-hr infusions (three patients) varied from 0.02 to 0.37 μg/ml, and during the 6-hr infusions (three patients) varied from 0.02 to 0.61 μg/ml. These results indicate that concentrations found to exhibit minimum inhibitory effects in in vitro cell studies are achievable during prolonged iv infusions of AZQ. [Cancer Treat Rep 69:1383–1385, 1985]

Diaziquone [(2,5-diaziridinyl-3,6-biscarboethoxyamino-1,4-benzoquinone) (AZQ)] is a new antitumor agent currently undergoing clinical trials. Clinical responses in patients with CNS malignancies have been favorable, with an overall response rate as high as 44% (1-3). Dosage schedules of AZQ have been quite variable, eg, 6-8 mg/m^2 daily for 5 days, repeated every 4 weeks; 15 mg/m^2 daily for 3 days, repeated every 4 weeks; 20 mg/m^2 on Days 1 and 8 of a 4-week cycle; and 20 mg/m^2 weekly for 4 weeks (1-5).

The objective of this study was to characterize the pharmacokinetics of AZQ in patients receiving the drug by three different dosage regimens. This would provide data about plasma concentrations of the drug associated with the different dosage regimens. Such information might prove useful for the design of future dosage schedules.

METHODS

Nine patients with histologic evidence of a primary brain neoplasm, and both clinical and radiologic evidence of tumor progression, participated in this study. The clinical response to AZQ treatment in these nine patients and in 18 others is described elsewhere (3). The drug was administered as continuous iv infusion using three different dosage regimens. The first three patients received a dose of 8 mg/m^2 daily for 5 consecutive days, administered as a continuous iv infusion over 20 minutes. The other six patients received a dose of 15 mg/m^2 daily for 3 consecutive days, where one-third of the dose was infused over 20 minutes and two-thirds of the dose was infused over either 4 hours (three patients) or 6 hours (three patients). Multiple blood samples were collected during and after the infusions on the first day of each treatment course. Serum samples were stored frozen at -20°C until analyzed.

AZQ for human use was supplied by the Investigational Drug Branch of the National Cancer Institute. Serum concentrations of AZQ were measured by means of a sensitive and specific high-performance liquid chromatographic (HPLC) method (6). Calibration curves covering the concentration range of 50-2000 ng/ml were constructed for each series of unknown samples. The lower

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limit of sensitivity of this assay was approximately 20 ng/ml. Average coefficient of variation of the calibration curves used during this study was 7%.

The pharmacokinetic parameters calculated were the elimination half-life ($t_{½\beta}$), total clearance (Cl), and apparent volume of distribution based on the area under the concentration versus time curve (Vd_{area}). In addition, apparent volume of distribution at steady-state (Vd_{ss}) and mean residence time (t) were calculated for the patients receiving the drug by a single 20-min infusion. These parameters were computed using conventional methods (7). Results are expressed as mean ± SD.

RESULTS

Peak serum concentrations after the rapid 20-min infusion of a dose of 8 mg/m² (0.193 ± 0.044 mg/kg) averaged 890 ± 314 ng/ml, ranging from 576 to 1203 ng/ml. Peak serum concentrations after the rapid 20-min infusion of one-third of a dose of 15 mg/m² (0.437 ± 0.236 mg/kg) ranged from 140 to 367 ng/ml, while serum concentrations during the subsequent slow 4-hr-long infusion of the remaining two-thirds of the dose averaged 81 ± 44 ng/ml, ranging from 22 to 153 ng/l. Peak serum concentrations after the rapid 20-min infusion of one-third of a dose of 15 mg/m² (0.302 ± 0.129 mg/kg) ranged from 112 to 608 ng/ml, while serum concentrations during the subsequent slow 6-hr-long infusion of the remaining two-thirds of the dose averaged 81 ± 44 ng/ml, ranging from 22 to 153 ng/ml. Peak serum concentrations after the rapid 20-min infusion of one-third of a dose of 15 mg/m² (0.302 ± 0.129 mg/kg) ranged from 112 to 608 ng/ml, while serum concentrations during the subsequent slow 6-hr-long infusion of the remaining two-thirds of the dose averaged 63 ± 21 ng/ml, ranging from 23 to 192 ng/ml. Figure 1 shows the ranges in serum concentrations of AZQ in the six patients who received the drug by the double-infusion method.

The pharmacokinetic parameters of AZQ are listed in table 1. There is no apparent difference in the kinetic parameters associated with the three dosage regimens, suggesting both dose- and time-independent kinetics. Vd_{ss} and $\bar{t}$ can only be reported for the single-infusion regimen. Vd_{ss} averaged 5.87 ± 2.73 L or 0.082 ± 0.016 L/kg, and $\bar{t}$ averaged 13.2 ± 6.7 mins.

DISCUSSION

The pharmacokinetic parameters of AZQ observed in this study are similar to those previously published (1,4,8). The elimination half-life values average about 0.5 hr, but ranged in this study from 12 to 59 mins. Total clearance has averaged about 0.4–0.5 L/min, but ranged in this study from 275 to 1034 ml/min, exhibiting about 3.8-fold variability when based on absolute values and about 3.3-fold variability when adjusted for body weight

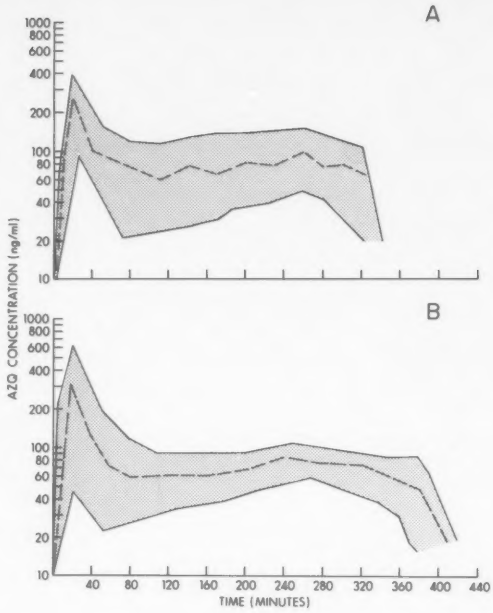

FIGURE 1.—AZQ concentrations in patients receiving a total dose of 15 mg/m², where ⅓ of the dose is administered over 20 mins and the remaining ⅔ of the dose is administered over either 4 hrs (A) or 6 hrs (B). The shaded areas represent the ranges in AZQ concentrations, whereas the stippled lines represent the average AZQ concentrations during these infusions.

(table 1). The apparent volume of distribution by area averaged 26.5 ± 14.7 L in this study, and 24.3 ± 5.7 L calculated from data presented by Schilsky et al (4). In contrast to the results from the present study and those by Schilsky et al (4), Bachur et al (8), and Curt et al (1), Lu et al (9) (using a different analytical methodology) revealed a terminal half-life of about 45 hrs, and Schilcher et al (5) (using an HPLC assay) reported a terminal half-life of about 17 hrs. It is difficult to reconcile these differences, since the former studies based on HPLC and spectrophotometric detection (present study, 1,4,8) would be expected (considering the sensitivity of the assay and the reported plasma levels) to detect the longer terminal elimination phase reported by the other groups (5,9). Total clearance calculated from these latter studies (5,9) averaged about 0.2 L/min, which is < 50% of the average total clearance reported in the other studies (present study, 1,4,8). The average plasma concentrations of AZQ observed during the prolonged infusions in the present study are in good agreement with the higher values for total clearance. One of the latter studies (9) involved the administration of radiolabeled AZQ; no data are available to indicate whether labeled drug is metabo-

TABLE 1.—Pharmacokinetic parameters of AZQ in patients

Patient No.	Dose (mg)	Infusion* (mins)	Weight (kg)	$t_{1/2}$ (mins)	Cl (ml/min)	Cl (ml/min/kg)	Vd_{area} (L)	Vd_{area} (L/kg)
1	10	20	69.9	59.1	319	4.56	27.2	0.389
2	10	20	44.5	39.0	275	6.17	15.5	0.348
3	13.6	20	64.5	33.0	964	15.0	45.9	0.713
4	9.8	240	14.5	52.7	353	24.3	26.9	1.855
5	14.8	240	72.6	35.9	1034	14.2	53.5	0.737
6	20	240	46.5	43.7	401	8.62	25.3	0.544
7	10	360	45.8	32.1	423	9.24	19.6	0.428
8	10	360	42.2	27.3	423	10.0	16.7	0.396
9	20	380	44.4	11.8	441	9.94	7.53	0.170
mean	13.1		49.4	37.2	515	11.3	26.5	0.620
SD	4.3		17.8	13.9	280	5.9	14.7	0.496

*The 20-min infusion was a single infusion, while the other 2 infusions, 240 and 360 mins, were double infusions where ⅓ of the dose was administered over the 1st 20 mins and the remaining ⅔ of the dose over either 4 or 6 hrs.

lized as rapidly as unlabeled drug.

Serum concentrations of AZQ during the prolonged infusion of two-thirds of a dose of 15 mg/m^2 averaged about 80 ng/ml over 4 hrs and 60 ng/ml over 6 hrs (fig 1). Concentrations in this range have been reported to be within the range of minimum inhibitory concentrations for inhibition of thymidine incorporation into L1210 cells and inhibition of the cloning of HL60 cells in soft agar (10). It remains to be established, however, whether different modes of AZQ administration, particularly with respect to rapid or prolonged infusions, are associated with differences in clinical efficacy and/or toxicity.

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Doxorubicin and Cisplatin Excretion Into Human Milk[1,2,3]

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Plasma and milk concentrations of doxorubicin (DOX) and cisplatin were measured after iv administration of these agents to a lactating patient with ovarian cancer. Cisplatin was undetectable in human milk. Milk concentrations of DOX often exceeded those detected in concomitant plasma samples. For DOX, the highest milk:plasma concentration ratio was 4.43:1 and was observed 24 hours after administration of the drug. The area under concentration versus time curve (AUC) for DOX was approximately the same in plasma and milk. Doxorubicinol was the major metabolite of DOX in plasma and in milk. The AUC for doxorubicinol was ten times higher in milk than in plasma. However, the total amount of anthracycline antibiotic delivered in the milk (maximum concentration of active anthracycline antibiotic: 0.24 mg/L) was negligible. [Cancer Treat Rep 69:1387–1389, 1985]

Although an infrequent occurrence, the administration of cytotoxic drugs to pregnant or lactating women is a difficult situation encountered in antineoplastic chemotherapy. In pregnant women, the decision of whether or not to use such agents is dictated by the relative benefits anticipated for the patient and the risks anticipated to the fetus (1–4). In lactating women, where direct fetal toxicity from transplacental transfer of drug is not a concern, it is prudent to terminate breast feeding. However, there is a paucity of information on the excretion into human breast milk and on concentrations of various antitumor drugs achieved in human breast milk, as w ll as on the danger to infants posed by ingestion of such milk. Doxorubicin (DOX) and cisplatin (DDP) are two commonly employed antineoplastic agents whose plasma pharmacokinetics are well described (5–16), but whose excretion into breast milk is uncharacterized. We recently had the opportunity to address this latter issue in a lactating woman with advanced ovarian carcinoma, a disease in which combination chemotherapy with DOX and DDP has significant activity (17).

MATERIALS AND METHODS

The patient was a 31-year-old female with stage II ovarian cancer who was 7 months postpartum when treatment was undertaken at the University of Massachusetts Medical Center. Her chemotherapy consisted of DOX at a dosage of 70 mg/m^2 (total dose, 90 mg) given on Day 1 as a 15-min iv infusion, and DDP at a dosage of 100 mg/m^2 (total dose, 130 mg) given on Day 1 as a 26-hr iv infusion. Milk and heparinized blood samples were obtained at 0.5, 1, 2, 5.25, 8, 14.25, 24, 48, and 78 hrs after DOX administration, or 0.25, 1.25, 4.5, 7.25, 13.5, 23.25, 47.25, and 71.25 hrs after the start of the DDP infusion. Plasma was obtained by centrifugation of blood samples and was stored frozen at -30°C until analyzed.

Daunorubicin was obtained from the Division of Cancer Treatment (National Cancer Institute, Bethesda, MD) and was shown to be chromatographically pure. Other chemicals were reagent grade. DOX and metabolites were extracted from plasma and were assayed by thin-layer chromatography as described by Benjamin et al (6). In

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milk, DOX and metabolites were assayed as follows: daunorubicin, as an internal standard (0.5 μM final concentration), was added to 1-ml samples of milk and the mixture was extracted with 2 ml of chloroform:isopropanol, 1:1 (vol:vol). After saturation with ammonium sulfate, the samples were centrifuged at 4°C for 10 mins at 27,000 × *g*. The organic phase was collected and dried under a nitrogen jet. The dried residue was redissolved in 100 μl of chloroform:isopropanol, 1:1 (vol:vol), and spotted onto 250-μM silica gel 60 thin-layer chromatography plates (E. Merck, Darmstadt, West Germany). The plates were developed, in an ascending fashion, in ethyl acetate for 16 cm, air dried, and then developed, in an ascending fashion, for 16 cm in chloroform:methanol:acetic acid:water, 80:20:14:6 (vol:vol:vol:vol). Fluorescent spots were identified under 254-nm light (UVS Mineralight, San Gabriel, CA), and were scraped from the plate. Fluorescence compounds were eluted from the silica gel with 2 ml of isopropanol:2.16 N sulfuric acid, 75:25 (vol:vol), and fluorescence was quantified with an Aminco-Bowman spectrofluorometer (S. L. M. Instruments, Inc, Urbana, IL) at an excitation wavelength of 470 nm and an emission wavelength of 550 nm. The assay in milk was linear between at least 0.01 μM and 1 mM.

Total platinum was assayed in plasma and milk by flameless atomic absorption spectrometry with the same methodology and instrumentation previously published (18). With this instrumentation and methodology, platinum recovery was 90%-100% and a sensitivity of 0.1 μg/ml was possible. Preliminary experiments showed no interference with the assay due to milk and linear recovery of platinum from a standard curve constructed in cow's milk.

Plasma and milk drug concentrations were modeled using the MLAB program (Division of Computer Resources and Technology, National Institutes of Health, Bethesda, MD) (19).

RESULTS

DOX was easily detectable in plasma. The peak concentration (1.51 μM) was observed at the first sampling time after initiation of the infusion (table 1). DOX disappeared from plasma with a terminal half-life of 52.9 hrs. The total-body clearance was 332 ml/min and the AUC was 8.3 μM × hr. Doxorubicinol (DOXOL) was the predominant metabolite in plasma. The AUC for this metabolite was 1.7 μM × hr. Small concentrations of other metabolites were observed, but their concentrations were too low for quantitation.

Plasma concentrations of platinum increased progressively during the infusion to reach a maximum value of 2.99 μg/ml. After discontinuation of the infusion, platinum concentrations decreased according to a single exponential decay with a half-life of 88.4 hrs. The AUC for platinum, including the infusion time, was 164.3 μg/ml × hr.

TABLE 1.—Pharmacokinetic parameters for DDP, DOX, and metabolites in plasma and milk

Parameters	DOX*	DOXOL	DDP
Plasma			
Peak concentrations	1.51 μM	0.15 μM	2.99 μg/ml
Time of peak (hrs)	0.5	0.5	24
AUC	8.3 μM × hr	1.7 μM × hr	164.2 μg/ml × hr
Total-body clearance (ml/min)	332	NA†	13.2
Terminal half-life (hrs)	52.9	26.2	88.4
Milk			
Peak concentration	0.24 μM	0.20 μM	ND‡
Time of peak (hrs)	24	24	NA†
AUC	9.9 μM × hr	16.5 μM × hr	NA†

*DOX and DDP were given to a lactating woman at a dosage of 70 mg/m² over 15 mins for DOX and 100 mg/m² over 26 hrs for DDP. Plasma and milk samples were obtained at specific times. DOX, metabolites (doxorubicinol:DOXOL), and DDP were assayed as described in Materials and Methods.

†NA=not applicable.

‡ND=not detected.

Milk concentrations of DOX increased to reach a maximum concentration of 0.24 μM at 24 hrs after treatment. At that time, the milk:plasma ratio was 4.4:1. The AUC for DOX in milk was 9.9 μM × hr. DOX was detectable in milk up to 72 hrs. DOXOL was also easily detectable in milk. The peak concentration of this metabolite (0.20 μM) was observed at 24 hrs after therapy. The AUC for DOXOL was 16.5 μM × hr. Platinum was undetectable at any time in milk.

DISCUSSION

The plasma pharmacokinetic parameters observed in this patient for DOX and DDP fall well into the range of those published in the literature (5-16). DOX concentrations declined biexponentially and DOXOL was the predominant plasma metabolite, as reported by others (5-8). Significant concentrations of DOX and DOXOL were detected in breast milk. Peak concentrations were observed 24 hrs after treatment and were almost five times as high as the concomitant plasma concentrations. Drug exposure, as measured by the AUC, was approximately the same for plasma and milk for DOX; however, for DOXOL, the AUC was almost ten times higher in milk than in plasma. Since DOXOL is felt to be an active metabolite of DOX, the exposure to active compound is higher in milk than in plasma. In contrast to DOX and its metabolites, no platinum was detected in milk.

The differential of transfer of DOX and DDP from plasma to milk may be related to their relative lipid solubility, protein binding, molecular weight, ionization, and possibly other factors. Although DOX and its metabolites

are present in milk, the total amount delivered to a nursing infant would be very small. The maximum concentration of DOX and DOXOL measured in milk was 0.24 μM (128 ng/ml) and 0.204 μM (111 μg/L), respectively. In conclusion, DOX and several metabolites are present in breast milk after treatment with DOX. However, the total amount of active anthracycline antibiotics that would be absorbed by a nursing child is small. In addition, DDP is not detected in breast milk. Although infant exposure to DOX and DDP from ingestion of breast milk would be very low, we still feel it prudent to advise lactating women receiving chemotherapy with these antineoplastic drugs to refrain from breast-feeding their infants.

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Cellular Basis for the Inefficacy of 5-FU in Human Colon Carcinoma[1,2]

Benjamin Drewinko* and Li-Ying Yang[3,4]

Five established human colorectal carcinoma cell lines with distinct phenotypic properties were exposed to different concentrations of 5-FU for varying time intervals. Effects were measured by sequential cell counts and by inhibition of colony formation. Treatment for 1 hr with 1 μg/ml barely decreased survival of all cell lines as measured by colony formation; at a concentration of 100 μg/ml, survival was modestly reduced for all cell lines, and for concentrations of 1000 μg/ml, survival was decreased by > 50%. Extending the length of the treatment interval markedly increased the degree of cell kill for all concentrations of 5-FU. Treatment for > 24 hrs resulted in almost complete extermination of colony-forming cells, even for relatively resistant cell lines. The effect of 5-FU treatment on cell number was more complex and depended on drug concentration, length of treatment, and type of cell line. In general, decrements in cell numbers were somewhat related to both drug concentration and length of treatment interval, especially if performed 7 days after terminating drug treatment. Earlier cell counts were inconclusive and the same result could be obtained for different drug concentrations or treatment intervals. Furthermore, these results would change on a daily basis. More important, cell count results never correlated with the survival endpoint measured by inhibition of colony formation. Our results suggest that enhancement of the currently poor performance of 5-FU in the treatment of human colon carcinoma could originate from changing the administration modality to long-term infusion. [Cancer Treat Rep 69:1391–1398, 1985]

The fluorinated pyrimidine 5-FU plays a central role in every chemotherapeutic scheme for treating colorectal cancer and serves as the standard reference for potential alternative antitumor agents for this disease. Yet, the overall response rate to this drug has remained < 25%, and its use has not significantly improved the survival of patients with large bowel cancer (1-6). Therefore, most patients with colorectal cancer receive a very toxic drug to which their tumor cells are relatively insensitive (3), at least at the dose levels and with the administration modes currently used. Similarly, combinations of 5-FU with other antitumor compounds have failed to improve significantly the results obtained with 5-FU alone, and even the apparent improvement in response rate attributed to some of these programs was not associated with increased survival of patients (5,6).

These considerations seriously challenge the usefulness of 5-FU as an antitumor agent for the average patient with colorectal cancer (3,5,7). The reason why this agent is even considered in antitumor therapy schemes for this disease is that no other drug, combination of drugs, or new means of administration has shown a clear advantage over results obtained with the fluorinated pyrimidine (1-8). Thus, 5-FU has acquired an exalted and conspicuous role in the treatment of colorectal cancer, not by distinguished clinical performance, but solely by default.

In contrast to such dismal clinical results, there is a plethora of preclinical experimental data documenting the antiproliferative activity of 5-FU. Obviously, a marked disparity exists between preclinical experimental results with 5-FU and subsequent clinical experience. Significantly, most spectacular results reported

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FU were obtained in experimental systems that had very little in common with human colorectal tumors (9-11); when 5-FU was evaluated on transplantable murine colonic cancer, the responses were much more modest and thus similar to those of actual clinical results (12,13).

We have evaluated the cytotoxic activity of 5-FU in an alternative system which we believe approximates more closely the desired target of clinical chemotherapy (14), while simultaneously using more relevant endpoints to determine cell lethality (15,16). We treated five established human colon carcinoma cell lines that have distinct phenotypic characteristics (ie, morphological differentiation, chromosome number, carcinoembryonic antigen production, etc) with varying concentrations of 5-FU that were presented to the cells for different periods of time. The activity of 5-FU at the cellular level was measured by sequential cell counts and by inhibition of colony formation capacity. Only modest degrees of actual cell killing were observed after short-term treatment, regardless of target cell line. Only when the concentration was increased to exceedingly high levels or when the exposure interval was prolonged did 5-FU induce an acceptable degree of cell kill.

MATERIALS AND METHODS

Cell Lines

Studies were performed on five permanently established human colorectal cancer cell lines representing three biological groups with distinct phenotypic properties (14). Group 1 consisted of two lines designated LoVo and SW 48; Group 2 comprised two lines called SW 480 and SW 620; and Group 3 was represented by line SW 403. All of these lines have been certified to be free of contamination with other established human cell lines (17-19). LoVo cells, established at The University of Texas M. D. Anderson Hospital in 1972, are propagated as monolayer cultures in Ham's F-10 medium supplemented by 20% fetal bovine serum, vitamins, glutamine, and antibiotics. All of the SW lines (SW 48, 403, 480, and 620) were obtained from the Scott and White Clinic, Temple, TX, and maintained in L-15 medium supplemented with 10% fetal bovine serum, glutamine, and antibiotics. Growth kinetics properties of each cell line have been previously described (14). Of particular interest to this article, doubling times (in hrs) for each cell line are as follows: LoVo, 37; SW 48, 46; SW 480, 45; SW 620, 44; and SW 403, 69.

Drugs

5-FU was obtained from Hoffmann-La Roche Laboratories (Nutley, NJ) as a dry powder and freshly dissolved for each experiment in 0.9% NaCl solution. Serial dilutions were prepared in the corresponding nutrient medium supplemented by dialyzed fetal bovine serum. The pH was adjusted, if necessary, to 7.2-7.4. The same nutrient medium, without drug, was used for control cultures.

Survival Assay

Stock cultures from each cell line were harvested by previously reported standard procedures and counted with the aid of an electronic particle counter (Coulter Counter Model ZBI, Coulter Electronics, Inc, Hialeah, FL). Cell suspension aliquots were seeded into 60-mm Petri dishes (5×10^5 cells/dish) and incubated at 37°C in air containing 5% CO_2 for the corresponding length of time to achieve exponential growth (14). The medium was discarded, and the cells were exposed to increasing drug concentrations (1, 10, 100, and 1000 μg/ml) at 37°C for the desired incubation interval (1, 6, 24, 48, and 72 hrs). The drug was decanted, and the cells were washed twice in Hank's balanced salt solution, harvested as a monodispersed suspension, and counted in duplicate. Known aliquots were dispensed into 60-mm Petri dishes so that 50-100 colonies would appear after a 21-day incubation in a 5% CO_2 humidified atmosphere at 37°C. Colonies were stained with 2% crystal violet in 95% ethanol. Viability was defined as the ability of single cells to originate a colony of $\geq$ 50 cells. For each experiment, the cloning efficiencies of at least six control cultures were assessed simultaneously, the cell counts of which served to determine the control growth curve. Control cultures consisted of cells treated in exactly the same manner as the test cells but without receiving the drugs. The survival fractions for the different drug concentrations were normalized for the individual controls of each experiment. All experiments were repeated at least twice with triplicate samples for each drug concentration. Growth curves for control and treated cells were determined for the week ensuing termination of drug treatment interval (ie, 48-hr treatment plus 7 days of cell counts for a total of 9 days) by decanting the drug and replacing with fresh nutrient medium. Cell counts were made in duplicate every 24 hrs for duplicate cultures.

RESULTS

Tables 1-5 present the effect of treating each cell line with increasing concentrations of 5-FU for varying periods of time. Effects are expressed both as reduction in cell number with respect to untreated controls (for the 7-day period immediately following termination of drug treatment) and as inhibition of colony formation measured 21 days after terminating drug treatment.

TABLE 1.—Effects of treating LoVo cell line with 5-FU*

5-FU concentration (μg/ml)	Treatment duration (hrs)	Cell counts (% of control)					% survival measured by colony formation
		Time after release of drug treatment					
		24 hrs	48 hrs	72 hrs	96 hrs	7 days	21 days
1	76.6	107	100	114	104	100	100
	6	83.7	78.3	77	117	NA	92
	24	45.2	69.2	58.5	96.8	> 100	52
	48	63.3	75.8	60.9	44.0	86.5	54
	72	NA	79.5	56.6	48.0	100	45
10	1	53.2	85.2	77.2	54.1	69.6	100
	6	62.7	60.0	69.8	70.8	45	47
	24	43.5	49.0	41.7	64.5	NA	5.1
	48	51.0	48.4	37.4	28.8	58.7	7.0
	72	67.9	46.2	38.8	29.7	56.3	0.2
100	1	42.6	63.0	53.5	53.6	42.6	75.3
	6	65.1	76.7	93.0	NA	31.3	3.7
	24	74.2	55.8	29.8	29.0	21.3	0.01
	48	38.8	16.5	8.0	4.0	4.4	NC
	72	17.0	7.9	3.6	1.7	3.1	NC

*NA = not available; NC = no colonies.

Data represent mean values of a single representative experiment, with various replicates for the different experimental procedures. Additional independent experiments yielded similar results. Treatment with 1 μg/ml for 1 hr barely decreased survival of all cell lines as measured by colony formation. At a concentration of 10 μg/ml (1 hr), cells from Group 1 and 2 were again minimally affected, whereas the survival of SW 403 cells (Group 3) was reduced by > 75%. At a concentration of 100 μg/ml (1 hr), survival was reduced for all cell lines with the maximal effect (8.5% survivors) again noted for SW 403 cells. For concentrations of 1000 μg/ml (data not shown), treatment for 1 hr decreased survival to the following values: LoVo, 12.7%; SW 48, 30%; SW 480, 13%; SW 620, 74%; and SW 403, 4.3%.

Extending the length of the treatment interval markedly increased the degree of cell kill for all concentrations of 5-FU. For cells of Group 1 and 2, treatment with 1 μg/ml for > 48 hrs decreased survival to < 50%, while that of SW 403 cells was reduced by > 99%. Similar results, albeit of a greater magnitude, were observed for treatment with 10 μg/ml. Again, SW 403 cells were the most sensitive for concentrations > 100

TABLE 2.—Effects of treating SW 48 cell line with 5-FU*

5-FU concentration (μg/ml)	Treatment duration (hrs)	Cell counts (% of control)					% survival measured by colony formation
		Time after release of drug treatment					
		24 hrs	48 hrs	72 hrs	96 hrs	7 days	21 days
1	1	93	> 100	100	110	108	75
	6	99.7	97.1	100	99.7	88.4	68
	24	96.3	100	100	NA	85.6	40
	48	65.9	40.1	53.3	58.1	NA	23
	72	51.4	42.0	33.0	22.6	21.7	11
10	1	102	109	100	96.7	95.3	67
	6	91	66.8	60.1	48.9	46.3	50
	24	71.6	57.7	34.0	NA	15.3	21.5
	48	57.9	40.0	27.0	12.6	11.0	6.1
	72	36.7	27.5	20.0	12.2	7.0	4.0
100	1	89.6	70.4	55.7	32.8	18.0	64
	6	87.9	41.6	40.2	30.6	9.6	36
	24	69.8	42.5	17.3	NA	2.5	1.0
	48	13.9	7.3	5.1	0.8	0.7	NC
	72	2.3	1.8	1.25	0.7	0.4	NC

*NA = not available; NC = no colonies.

TABLE 3.—Effects of treating SW 480 cell line with 5-FU*

5-FU concentration (μg/ml)	Treatment duration (hrs)	Cell counts (% of control)					% survival measured by colony formation
		Time after release of drug treatment					
		24 hrs	48 hrs	72 hrs	96 hrs	7 days	21 days
1	1	> 100	> 100	100	90	> 100	100
	6	70.6	91.3	90.5	123	115	100
	24	50.0	33.3	24.0	32.1	75	34
	48	28.6	25.6	17.1	28.0	32.7	2.3
	72	56.5	55.4	54.7	50.0	34.3	2.1
10	1	> 100	89.3	52.3	49.0	> 100	100
	6	51.8	37.4	28.6	45.5	> 100	91
	24	30.0	22.2	19.0	25.0	43.8	2.0
	48	21.4	22.8	24.3	12.5	23.6	NC
	72	40.3	42.6	42.9	47.1	25.7	NC
100	1	85.7	48.6	20.5	14	56.7	32.2
	6	38.2	28.3	20.5	26.4	30.3	0.03
	24	18.0	11.1	9.0	10.0	17.0	NC
	48	5.2	2.0	5.0	6.0	3.1	NC
	72	8.9	11.1	14.3	17.7	8.6	NC

*NC = no colonies.

and 1000 μg/ml. Treatment for > 24 hrs resulted in almost complete extermination of colony-forming cells, even for the relatively resistant SW 620 cells.

The effect of 5-FU treatment on cell number was more complex and depended on drug concentration, length of treatment, and type of cell line. For cells in Groups 1 and 2, treatment with 1 μg/ml for 1 hr resulted in either an initial decrease in cell number promptly restored to control levels by 48 hrs or no change at all. Only for the sensitive SW 403 line did cell numbers decrease steadily. Increasing the length of the treatment interval produced early decreases in cell numbers (24-72 hrs following treatment) with subsequent rebounds to control, or nearly control values, for the less sensitive cells or for the shorter treatment intervals. In other cases (longer treatment intervals or more sensitive lines), cell numbers remained at a uniformly low level with respect to control during the experimental interval, or steadily declined with increasing posttreatment time. Increasing the concentration of 5-FU to 10 and 100 μg/ml accentuated these changes in cell numbers with a variety of different results depending on cell type, drug concentration and length of incubation, and posttreatment day when cell count was per-

TABLE 4.—Effects of treating SW 620 cell line with 5-FU*

5-FU concentration (μg/ml)	Treatment duration (hrs)	Cell counts (% of control)					% survival measured by colony formation
		Time after release of drug treatment					
		24 hrs	48 hrs	72 hrs	96 hrs	7 days	21 days
1	1	> 100	> 100	> 100	> 100	97.8	80
	6	80	110	89.8	89.2	> 100	89.6
	24	90	61.4	50.0	57.1	100	80
	48	51.1	35.3	31.3	30.4	79.6	61
	72	26.5	25.0	23.2	25.0	27.1	27
10	1	> 100	> 100	> 100	> 100	80.4	92
	6	83.3	69	42.9	35.1	44.4	90
	24	72	68.1	33.6	30.0	20.0	53
	48	33.3	23.5	20.6	19.0	20.4	17.1
	72	14.7	10.3	7.4	5.6	25.0	1.3
100	1	26.7	104	58.8	42.2	14.7	81.5
	6	56.0	41.4	31.6	24.6	18.9	74.2
	24	44.0	29.5	20.3	20.0	13.3	10.5
	48	17.8	12.6	10.5	8.7	6.1	0.35
	72	10.0	7.0	4.8	4.0	NA	NC

*NA = not available; NC = no colonies.

TABLE 5.—Effects of treating SW 403 cell line with 5-FU*

5-FU concentration (μg/ml)	Treatment duration (hrs)	Cell counts (% of control)				% survival measured by colony formation
		Time after release of drug treatment				
		24 hrs	48 hrs	72 hrs	96 hrs	21 days
1	1	93.9	66.4	80.7	55.7	77
	6	NA	NA	NA	NA	NC
	24	44.4	34.7	24.4	18.5	6.3
	48	21.7	14.6	12.1	10.5	NC
	72	16.0	12.1	10.5	8.9	0.74
10	1	71.2	45.4	39.1	16.9	23.2
	6	NA	NA	NA	NA	NC
	24	43.5	23.5	18.0	NA	3.7
	48	20.1	17.5	15.0	12.4	1.8
	72	17.4	15.5	12.5	12.4	NC
100	1	68.2	48.0	22.0	8.9	8.5
	6	NA	NA	NA	NA	NC
	24	39.5	28.2	18.0	NA	1.6
	48	12.7	6.9	8.5	NA	NC
	72	10.3	8.5	7.5	5.9	NC

*NA = not available; NC = no colonies.

formed. In general, decrements in cell numbers were somewhat related to both drug concentration and length of treatment interval, especially if performed 7 days after terminating drug treatment. Earlier cell counts were inconclusive, and the same result could be obtained for different drug concentrations or treatment intervals. Furthermore, these results would change on a daily basis, preventing a definite conclusion concerning the activity of the agent. More important, cell count results never correlated with the survival endpoint measured by inhibition by colony formations. Thus, the ratios of treated cell counts with respect to control cells could be either considerably above or markedly below the eventual decrease in colony formation capacity, and only in very rare instances approximated the final colony count.

DISCUSSION

Most evaluations reporting great efficacy of 5-FU have been conducted in bacteria, on noncolonic rodent tumors, or on noncolonic human cell lines (9,10,20). When 5-FU was used to treat transplantable murine colon carcinoma models, much less spectacular results were observed (12,13). In agreement with the fact that human cells are usually less sensitive than rodent cells to the effects of many antitumor agents (21–27), when 5-FU treatment was applied to human cells very little lethal activity was observed, and then only at relatively high concentrations and after prolonged incubation intervals (25,28–43).

Differences in dose levels and treatment intervals could be major factors in the disparity between experimental observations and clinical responses. Cell kill responses induced by low concentrations of 5-FU require treatment intervals approaching or exceeding the generation time of the target cells (28,29,32,33,35, 41–48). For transplantable murine tumors, including colon carcinomas, closely spaced administrations of 5-FU doses that may range up to 300 mg/kg are required for significant inhibitions of tumor growth or increased life spans (9,11,12,49–51). Yet, the accepted clinical practice is a weekly bolus administration dose of 15 mg/kg (8). Although the predictive value of pharmacokinetic data concerning 5-FU levels in plasma is uncertain (52), it is interesting that conventional bolus doses produce initial peak plasma levels of 10^{-4} to 10^{-3} M (about 50–100 μg/ml) which rapidly (10–15 mins) and nonlinearly fall to levels of 10^{-8} M (40–42,53–57). These concentrations are clearly several orders of magnitude higher than the 0.5–1 μg/ml values that produce in vitro biochemical and growth-inhibiting effects in *continuous treatment*. Consequently, it seems reasonable that the limited success of 5-FU in human oncotherapy is due, in part, to drug levels that are insufficient to produce significant degrees of tumor cell kill, possibly because of the slow and mediocre uptake of the drug by the target cells (9,29,30,41–43,58,59) which is thus unavailable throughout the length of their cell cycle, a necessary condition for cell kill (41,43).

The results presented in this manuscript confirm our previous report (43) and those of others (32–38, 40,42,60,61) demonstrating the relative insensitivity of human colon carcinoma cells, both fresh tumor samples and established cell lines (WiDr; SW 480; Cx1; Colo 205 and 320; AGS1, 4 and 6; HC 219; HT-29; HCT-8; and BE), to treatment with 5-FU unless delivered in either

high concentrations for short treatment intervals or, preferably, in continuous treatment. Our data were obtained on five different human colon carcinoma cell lines with distinct phenotypic expressions of which only one (LoVo) received 5-FU treatment in the presence of negligible amounts of thymidine (0.7 μg/ml) contained in the Ham's F-10 medium. However, we and others have shown that this concentration of thymidine is insufficient to protect cells from the lethal effects of the drug (30,39,42,43,62-66). All of the other lines were treated in L-15 medium, which does not contain TdR. Furthermore, cell lethality was determined by inhibition of colony formation, as cell counts were clearly influenced by 5-FU-induced cycle progression delay in accordance with the experience of others (60,61), and could not be correlated with abrogation of reproductive integrity. Under these conditions, treatment for 1 hr with 1 μg/ml of 5-FU failed to induce any significant degree of cell kill. At 10 μg/ml, treatment for 1 hr showed only one sensitive cell line (SW 403), while at 100 μg/ml, all lines showed considerable degrees of cell kill. However, these latter concentrations are much too high in comparison to those clinically achievable. Yet, even at concentrations of 1 μg/ml, extending the incubation interval for as little as 24 hrs produced spectacular and dramatic decreases in cell survival for all cell lines. Interestingly, continuous iv infusion of 5-FU will attain levels of 0.5-1 μg/ml of plasma for several days with tolerable degrees of myelotoxicity (67-71). According to our data, these conditions would be clearly sufficient to produce degrees of cell kill > 50% for most human colon cancers if the treatment interval were sufficiently prolonged. Thus, the failure of obtaining better responses in early clinical studies comparing bolus versus infusion of 5-FU (72,73) could be ascribed to the very short time of the infusion (8 and 2 hrs, respectively).

It seems clear that if 5-FU is to have any further impact in the treatment of colon carcinoma, different strategies in the administration of this agent must be developed. Thus, the current evaluations of long-term infusions of 5-FU (68,69,74-76) appear as the only rational mode of delivery on the basis of toxicologic studies, preliminary clinical responses, pharmacokinetic evaluations, and cellular responses to the drug. Whether these considerations will actually translate into improved approaches to the treatment of colon cancer remains to be demonstrated.

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1,25-Dihydroxyvitamin D_3: In Vivo and In Vitro Effects on Human Preleukemic and Leukemic Cells[1,2]

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The active metabolite of vitamin D known as 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is a major physiologic regulator of mineral metabolism in man. The compound is also a potent inducer of differentiation of a human promyelocytic leukemia cell line known as HL-60. The induction of differentiation of myeloid leukemia cells to functional end cells offers an appealing therapeutic prospect. We investigated the ability of $1,25(OH)_2D_3$ both to induce in vitro the differentiation of blast cells taken from patients with acute myelogenous leukemia and to improve hematopoiesis in vivo in patients with the myelodysplastic syndromes (preleukemia). We found that high concentrations (10^{-6} M) of $1,25(OH)_2D_3$ significantly induced the in vitro differentiation of blast cells as measured by morphology, phagocytosis, and superoxide production. A concentration of 10^{-9} M $1,25(OH)_2D_3$ had no effect on blast cell differentiation. We gave 2 μg/day of $1,25(OH)_2D_3$ to 18 patients with myelodysplastic syndrome (preleukemia) in an attempt to improve their hematopoiesis. During therapy, their peak peripheral blood granulocyte, platelet, and macrophage concentrations were slightly elevated as compared to their baseline, starting levels. Eight patients had a partial or minor peripheral blood response to the compound during the administration of $1,25(OH)_2D_3$. However, no patient showed significant improvement of peripheral blood cell or marrow blast cell counts by the end of the study (≥ 12 weeks) as compared to their starting levels. Seven of the patients developed leukemia before or by 12 weeks of treatment. Nine of the 18 patients developed hypercalcemia. Taken together, the study shows that high concentrations (10^{-6} M) of $1,25(OH)_2D_3$ can induce differentiation of leukemic blast cells in vitro, but the administration of $1,25(OH)_2D_3$ to patients with the myelodysplastic syndromes (preleukemia) does not have an enduring therapeutic effect. Hypercalcemia prevented administering greater amounts of $1,25(OH)_2D_3$. In the future, the use of new vitamin D analogs that induce hematopoietic cell differentiation without inducing hypercalcemia might allow the achievement of higher blood levels of the inducing compound and might be medically useful for selected preleukemic and leukemic patients. [Cancer Treat Rep 69:1399–1407, 1985]

Acute myelogenous leukemia (AML) often arises from neoplastic transformation at the pluripotent stem cell level. A block in cell maturation frequently occurs at the recognizable myeloblast or promyelocyte stage of differentiation. The leukemic patient often dies of infection or hemorrhage because the blast cells cannot mature to functional, terminally differentiated cells. Instead, many of the leukemic cells remain in the proliferative pool and rapidly accumulate.

Myelodysplastic syndromes (preleukemia) represent a spectrum of hematopoietic dysfunctions that can evolve to acute leukemia. Morphologic, karyotypic, enzymatic,

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and kinetic data indicate that preleukemia results from neoplastic transformation at a pluripotent stem cell level (1-3). The preleukemic clone has a selective growth advantage over the normal stem cells. This is reflected in vitro by a delay in maturation and in vivo by peripheral blood cytopenias and ineffective hematopoiesis in the marrow (1-4).

Unlike AML, however, substantial numbers of mature myeloid cells may be present in the peripheral blood of patients with preleukemia. Many of these mature-appearing cells are derived from the neoplastic clone (4,5). Preleukemia may be conceived as an early phase of leukemia where the neoplastic clone is established and manifested functionally by partially effective hematopoiesis. Precursor maturation becomes progressively impaired with termination in a severe maturational block characteristic of AML.

The life-threatening cytopenias of both preleukemia and leukemia might be prevented if cells in the neoplastic clone could be induced to mature (6). Studies using several murine and human AML cell lines showed that a variety of nonphysiological and several physiological agents could induce leukemia blast cells to differentiate to functional, mature cells (6-10).

The seco-steroid known as 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] can induce cells from both the murine myeloid leukemia line (M-1) (11) and the human promyelocytic leukemia line (HL-60) (6,13-15) to differentiate to monocyte-macrophage-like cells. Both the M-1 and HL-60 cells, when cultured with $1,25(OH)_2D_3$ (10^{-6} to 10^{-10} M), become adherent to charged surfaces, develop long filamentous pseudopodia, express chemotactic receptors, and acquire the ability to phagocytose microorganisms. Development of leukemia is inhibited or the survival time of mice prolonged after M-1 leukemia cells are injected into syngeneic mice and the mice are treated with $1,25(OH)_2D_3$.

Experiments using murine and human leukemic cell lines suggest that blast cells of certain leukemias may undergo terminal differentiation with exposure to $1,25(OH)_2D_3$ (6). We studied both the ability of $1,25(OH)_2D_3$ to induce differentiation of fresh human AML cells in vitro and the ability of $1,25(OH)_2D_3$ taken orally to improve hematopoietic parameters in preleukemic patients.

METHODS AND PROCEDURES

In Vitro Study: Effect of $1,25(OH)_2D_3$ on Blast Cells From Patients With AML

Cells.—AML cells from nine patients with leukemia were studied in vitro. Five patients had acute myeloblastic leukemia (M1, M2), two had acute myelomonocytic leukemia (M4), one had myeloid blast crisis, and one had lymphoid blast crisis of chronic myelogenous leukemia (CML). The patients were morphologically and histochemically classified by criteria developed by the French-American-British (FAB) Cooperative Group (16). The cytochemical stains performed on the peripheral blood and bone marrow of the patients included Wright-Giemsa, α-naphthyl acetate esterase (NAE), myeloperoxidase (MPO), Sudan black, ASD-chloroacetate esterase, and periodic acid-Schiff (17,18). Patients 1, 3, and 5 had converted from preleukemia to AML. During their preleukemia phase, these patients received $1,25(OH)_2D_3$ by mouth. These patients have the same reference numbers in the in vitro and in vivo studies. All patients had high peripheral blood leukemic cell counts, and 10-20 ml of heparinized venous blood was drawn from these appropriately informed patients. The mononuclear blood cells were isolated by buoyant density centrifugation (19); the nonadherent cells were obtained by twice incubating the cells on plastic for 1 hour at 37°C in culture medium (*see* below) in 7.5% CO_2, humidified air, and by collecting the final, nonadherent cells. The cell suspensions contained ≥ 86% blast cells (median, 92%) as judged by morphology of Wright-Giemsa-stained cytocentrifuge preparations.

Cell growth and maturation.—Five-hundred thousand peripheral blood, nonadherent mononuclear cells per milliliter was placed in 175-cm^2 flasks (Falcon Lab-ware, Division Becton Dickinson, Oxnard, CA) with alpha medium (Flow Laboratories, Inc, Rockville, MD) containing 10% fetal calf serum, penicillin/streptomycin at 37°C in 7.5% CO_2, and air in a humidified incubator. Cultures were harvested on Day 6 for viable and differential cell counts, and cytochemical and functional analysis. Viable cell counts were performed using trypan blue exclusion. Cytocentrifuge preparations were made and the histochemical stains Wright-Giesma, MPO, and NAE were used to determine cell lineage (17,18). Morphologic classification of cellular maturation in the granulocytic and monocytic series was as follows: immature cells included blasts, progranulocytes, and promonocytes; mature cells included myelocytes, metamyelocytes, bands, polymorphonuclear neutrophils, and macrophages. The method to study phagocytosis of *Candida albicans* and reduction of nitroblue tetrazolium (NBT) was described in detail (20,21).

Administration of $1,25(OH)_2D_3$ to Myelodysplastic (Preleukemic) Patients

Eighteen patients had myelodysplastic syndrome as determined by the FAB classification (22). All patients were anemic and had megaloblastic erythropoiesis. All patients had abnormal megakaryocytes and/or disorderly granulopoiesis (1-3). The patients were not deficient in vitamin B_{12} or folate and had not received cytotoxic therapy. All patients had adequate liver and renal function (bilirubin < 2.5 mg/dl) and a Karnofsky performance status of ≥ 60. All patients received a ≥ 3-week trial of

high-dose (> 150 mg/day) pyridoxine prior to entering the study. None of the patients had a hematopoietic response to the pyridoxine. Patients were questioned and examined prior to initiation of the trial concerning hypo- or hyper-parathyroidism; gastrointestinal abnormalities associated with malabsorption; acromegaly; Cushing's syndrome; hypopituitarism; sarcoidosis; evidence of vitamin D deficiency; renal lithiasis; previous history of hypercalcemia; and history (within the previous 4 months) of ingestion of either "megadoses" of vitamins, especially vitamin D, systemic adrenocortical or anabolic steroids, Dilantin, barbiturates, estrogens, sodium fluoride, calcium supplements, or calcium-containing antacids. Patients responding affirmatively to any of the above questions were excluded from the study.

Patients were assigned to initially receive two 0.25-μg capsules of $1,25(OH)_2D_3$ (Rocaltrol) per day, one capsule administered at breakfast time and the other at dinner time. At the start of Week 2, the dose escalated to two capsules twice a day. At the start of Week 3, the dose escalated to three capsules twice a day, and starting on Week 4, increased to a maximum of four capsules twice a day (2.0 μg) for the remainder of the treatment period. The treatment period was ⩾ 12 weeks. Patients had an initial cbc; bone marrow examination; and biochemical tests, including glucose, electrolytes (Na^+, K^+, Cl^-, CO_2 content), BUN, creatinine, total protein, albumin, bilirubin, alkaline phosphatase, LDH, SGOT, calcium, phosphorus, magnesium, and folate and vitamin B-12 and iron/iron-binding capacity. Patients were followed in the clinic every week for the first 4 weeks and then at least every 2 weeks for the remainder of the study. At each visit, a physical examination, and an interval history, including transfusion requirements, were recorded. A cbc with differential, and platelet count and serum calcium levels were performed at each visit. Bone marrow biopsy, aspirate, and karotypic examination were performed before and at the end of the $1,25(OH)_2D_3$ trial.

Criteria of response.—Partial response was defined as a ⩾ 50% improvement in all peripheral blood hematologic parameters for a period of ⩾ 4 weeks or a ⩾ 50% reduction in bone marrow blast cell count at the end of the study. Minor response was defined as an objective improvement in disease, ie, improvement in peripheral blood hematologic paramenters for a period of ⩾ 4 weeks or a reduction of bone marrow blast cells but less than that required for a partial response. Stable disease was defined as no change in any hematologic parameter throughout the treatment period. Finally, progressive disease was defined as progression to acute leukemia or worsening of neutropenia, anemia, thrombocytopenia, or blood monocyte concentration.

1,25 $(OH)_2D_3$.—$1,25(OH)_2D_3$ for the in vivo and in vitro studies was a generous gift of Hoffmann-La Roche (Nutley, NJ). For in vitro studies, stock solution of $1,25(OH)_2D_3$ was prepared in ethanol at 10^{-4} M and stored at -70°C. The sterol was diluted to the required concentration in alpha medium at the time of culture. Control plates containing only the diluent had no effect on growth or differentiation when diluted to the concentration of ethanol present in the experimental culture plates. The vitamin D compound was protected from bright light.

Statistical Methods

To avoid the assumption of normality, nonparametric statistical methods were used for data analysis. For the in vitro study, time and dose effects on Day 6 (evaluated separately for each dose) were assessed using the Wilcoxon signed rank test. In the latter case, P values were adjusted for multiple-dose comparisons using Bonferroni's method. Overall dose effects on Day 6 were assessed using a repeated measures version of the Wilcoxon signed rank test which is sensitive against ordered alternatives (23).

For the in vivo study, the peak and end-of-study drug effects were assessed also with the Wilcoxon signed rank test adjusted for multiple time comparisons. Association between different variables was determined with Spearman's rank correlation coefficient.

RESULTS

Effect of $1,25(OH)_2D_3$ on Maturation of Human Leukemic Cells In Vitro

We examined the effect that $1,25(OH)_2D_3$ had on differentiation of freshly isolated myeloid leukemic blast cells from nine patients (fig 1). Blast cells of two patients (Nos. 1, 2) were at the early myeloblast (M1) stage of development; blast cells of three patients (Nos. 3, 4, and 5) were at the myeloblast stage (M4); cells from Patient 8 were CML myeloid blasts; and cells from Patient 9 were CML lymphoid blasts.

Net cellular proliferation was examined after the blast cells from each of the leukemic patients were exposed for 6 days to either $1,25(OH)_2D_3$ or culture media (control) (fig 1A). The median of Day 6 cell counts (10^5/ml) in flasks containing 10^{-6} M $1,25(OH)_2D_3$, 10^{-7} M $1,25(OH)_2D_3$, or culture median alone (control) was 4 (range, 2-8), 5 (range, 2-9), and 7 (range, 5-9), respectively. Analysis of the overall dose responses showed that a concentration of 10^{-6} M $1,25(OH)_2D_3$ in the culture flasks produced a significant inhibition of proliferation as compared to Day 6 control flasks ($P < 0.05$).

The $1,25(OH)_2D_3$ induced maturation of leukemic cells to monocytes, macrophages, metamyelocytes, and granulocytes. On Day 0, the median percent of immature myeloid cells (myeloblasts and promyelocytes) placed into culture was 95% (range, 86%-99%). On Day 6, the median percent of mature myeloid cells in flasks containing 10^{-6}

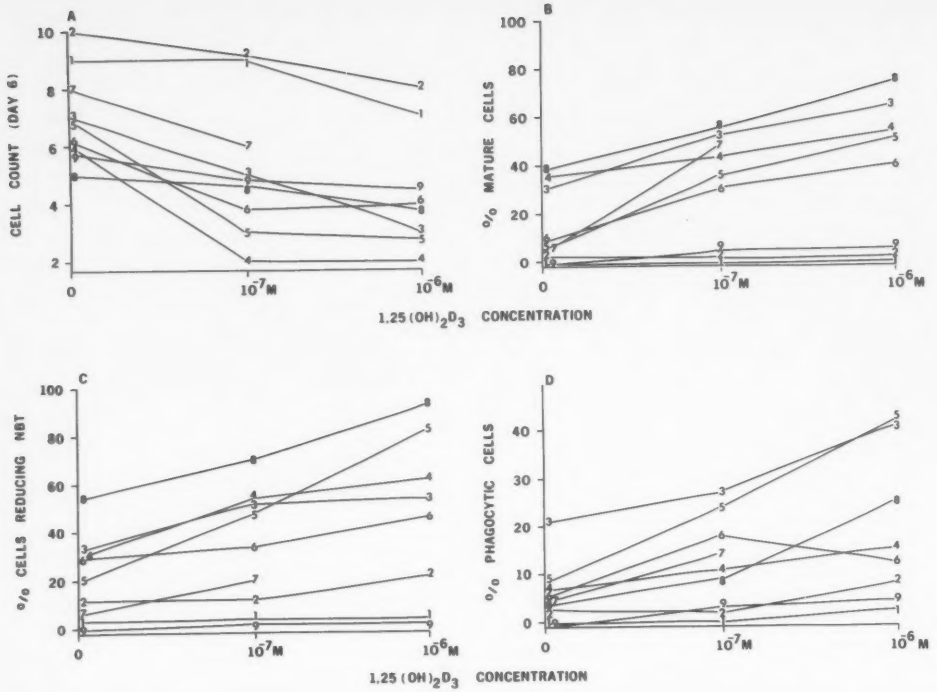

FIGURE 1.—Effect of exposure of $1,25(OH)_2D_3$ on proliferation and differentiation of blast cells harvested from patients with myeloid leukemia. **A:** effect of $1,25(OH)_2D_3$ on proliferation of leukemic blast cells (cell counts × 10^5/ml); **B**: effect of $1,25(OH)_2D_3$ on morphological maturation of leukemic blast cells; **C**: effect of $1,25(OH)_2D_3$ on NBT reduction by leukemic blast cells; and **D**: effect of $1,25(OH)_2D_3$ on *Candida* phagocytosis by leukemic blast cells. Cells were cultured in flasks containing 0, 10^{-6} M or 10^{-7} M $1,25(OH)_2D_3$ and were harvested on Day 6, when viable cell numbers, morphology, and function of the cells were determined. The patients are listed by No.

M $1,25(OH)_2D_3$, 10^{-7} M $1,25(OH)_2D_3$, or culture medium alone was 40%, 36%, and 5%, respectively (fig 1B). Cellular maturation in the control flasks did not significantly change ($P = 0.55$) as compared to the starting population. In contrast, the flasks containing either 10^{-7} M or 10^{-6} M $1,25(OH)_2D_3$ had a significantly greater percent of mature cells ($P < 0.05$) as compared to the starting cell population. Also, the percent of mature myeloid cells at Day 6 of culture was significantly ($P < 0.05$) higher for the flasks containing 10^{-6} M $1,25(OH)_2D_3$ as compared to Day 6 control flasks. Flasks containing 10^{-7} M $1,25(OH)_2D_3$ had an increased percent of mature cells, but were not significantly different from the Day 6 control flasks ($P = 0.054$).

Reduction of NBT is dependent on an intact respiratory burst with production of hydrogen peroxide (H_2O_2) and superoxide (O_2^-). Macrophages and granulocytes are able to reduce NBT but their immature progenitors cannot. We examined the ability of the leukemic cells from the nine patients to reduce NBT after culture with $1,25(OH)_2D_3$ for 6 days (fig 1C). The median percent of NBT-reducing cells in flasks containing 10^{-6} M and 10^{-7} M $1,25(OH)_2D_3$ or culture median alone was 49%, 33%, and 10%, respectively. The percent of NBT-reducing cells was significantly ($P < 0.05$) greater in the flasks containing 10^{-6} M $1,25(OH)_2D_3$ as compared to Day 6 control flasks. The flasks containing 10^{-7} M $1,25(OH)_2D_3$ did not contain a significantly ($P = 0.056$) increased percent of myeloid cells capable of reducing NBT as compared to cells in Day 6 control flasks.

A valuable function of normal mononuclear and polymorphonuclear phagocytes is the ingestion of foreign material. We examined the ability of the blast cells from the leukemic patients to phagocytose *Candida* after culture with $1,25(OH)_2D_3$ for 6 days (fig 1D). Median percentages of myeloid cells which phagocytosed *Candida* in flasks containing 10^{-6} M $1,25(OH)_2D_3$, 10^{-7} M $1,25(OH)_2D_3$, or culture medium alone was 14%, 11%, and 5%, respectively. The percent of phagocytic cells was significantly ($P < 0.05$) increased in the flasks containing 10^{-6} M $1,25(OH)_2D_3$ as compared to Day 6 control flasks. In contrast, the number of phagocytic cells was not signifi-

cantly different in flasks containing 10^{-7} M $1,25(OH)_2D_3$ as compared to Day 6 control flasks.

Taken together, the $1,25(OH)_2D_3$ (10^{-6} M) had a dose-related significant ($P < 0.05$) effect on the inhibition of cellular proliferation and the induction of maturation of leukemia cells as determined by morphology, NBT reduction, and phagocytosis ($P < 0.05$). Likewise, the blast cells from the more mature subtypes of AML such as M2 (Patients 3, 4, and 5) and M4 (Patients 6 and 7) were able to undergo greater maturation than the less mature leukemic subtypes [eg, M1 (Patients 1 and 2)] with exposure to $1,25(OH)_2D_3$. We examined the effect of 10^{-9} M $1,25(OH)_2D_3$ on proliferation and differentiation of the leukemic cells taken from the patients (Nos. 1-9) and found almost no change in growth or function of these cells as compared to cells from control flasks containing culture medium alone (data not shown).

Effect of Administration of $1,25(OH)_2D_3$ to Patients With Myelodysplasia (Preleukemia)

Eighteen myelodysplastic (preleukemic) patients were treated with $1,25(OH)_2D_3$ (table 1). Their ages ranged from 34 to 80 years, with a median age of 65. Eleven patients were male and seven were female. The median time from diagnosis of myelodysplasia to initiation of therapy with $1,25(OH)_2D_3$ was 7 months (range, 3-35). Seventeen of the 18 patients were anemic prior to treatment (median hematocrit, 24%; range, 21%-43%), and 14 of the patients received rbc transfusions every 4-8 weeks because of symptomatic anemia (table 2, fig 2). The median reticulocyte count prior to therapy was 1.5 (range, 0.2-4.7). Thirteen of 18 patients had thrombocytopenia prior to therapy, with a median of 58,000 platelets/μl of blood (table 2, fig 2). None of the patients received a platelet transfusion. The blood granulocyte count was depressed in 13 of the 18 patients prior to therapy (median, 2300/μl; table 2, fig 2). The pretherapy bone marrow of each patient was studied prospectively by a pathologist who was independent of the study and also by an investigator (H. P. Koeffler), and both agreed on the diagnosis of each patient. The median percent (range) of myeloblasts in the marrow and overall marrow cellularity was 10% (3%-25%) and 80% (20%-95%), respectively (table 2, fig 2). The marrow of each of the patients showed myelodysplasia; three patients had refractory anemia with excess myeloblasts (RAEM) in transformation, nine had RAEM, five had primary acquired sideroblastic anemia, and one had chronic myelomonocytic leukemia.

All patients received weekly escalating (0.5 μg) doses of $1,25(OH)_2D_3$ until a daily dose of 2 μg was reached. The median duration of therapy with $1,25(OH)_2D_3$ was 12 weeks (range, 4 to > 20). While on therapy, the granulocyte, monocyte, and platelet blood concentrations increased in most patients as compared to their starting values, and as a patient group, each of the peak blood values increased significantly as compared to beginning and ending blood values (table 2, fig 2). In contrast, the concentration of granulocytes, monocytes, and platelets in the blood was not significantly different between the be-

TABLE 1.—Clinical characteristics and effect of $1,25(OH)_2D_3$ on myelodysplastic (preleukemic) patients*

Patient No.	Age (yrs)/sex	Mos from diagnosis to treatment	Duration of treatment (wks)	Diagnosis before treatment	End of treatment	Response
1	70/F	4	13	RAEB-TR	AML (M1)	Progression
2	60/M	11	12	RAEB-TR	AML (M2)	MR; progression
3	59/M	20	12	RAEB-TR	AML (M2)	Progression
4	34/F	3	14	RAEB	AML (M4)	PR; progression
5	51/M	30	12	RAEB	AML (M2)	Progression
6	71/M	7	10	RAEB	AML (M4)	Progression
7	65/M	5	4	RAEB	AML (M1)	Progression
8	36/F	10	12	RAEB	RAEB	MR
9	66/F	6	15	RAEB	RAEB	MR
10	78/F	3	12	RAEB	RAEB	Stable
11	49/M	5	6	RAEB	RAEB	MR
12	80/M	6	7	RAEB	Death†	Progression
13	74/M	18	12	CMML	CMML	Stable
14	71/M	35	8	PASA	RAEB	Progression
15	65/F	27	12	PASA	PASA	MR
16	59/M	23	8	PASA	PASA	MR
17	52/M	7	12	PASA	PASA	Stable
18	61/M	12	> 20	PASA	PASA	MR

* RAEB-TR = refractory anemia with excess blasts in transformation; AML = acute myelogenous leukemia with M subclassification according to FAB classification (ref 16); RAEB = refractory anemia with excess blasts; CMML = chronic myelomonocytic leukemia; PASA = primary acquired sideroblastic anemia; MR = minor response; and PR = partial response.

†Death due to hemorrhage.

TABLE 2.—Treatment of preleukemic patients with $1,25(OH)_2D_3$*

	Median	Range	*P* value†
Granulocyte count (cells/μl of blood)			
Start	2300	180–11,520	
Peak	3750	486–29,820	0.0014
End	3110	96–24,480	0.0694
Macrophage count (cells/μl of blood)			
Start	50	3–8435	
Peak	300	43–16,898	0.0044
End	80	3–10,200	0.3156
Platelet count (x 10^3 cells/μl of blood)			
Start	58	12–300	
Peak	105	23–610	0.0006
End	65	4–358	1.00000
Marrow blasts (%)			
Start	10	3–20	
End	15	4–70	0.0136
Serum calcium (mg/dl)			
Start	8.9	8.2–10.4	
Peak	10.8	9.7–17.0	0.004
End	10.3	9.6–12.4	0.004

* Results represent data from 18 preleukemic patients. Start = initial value before therapy; peak = highest value during therapy; and end = value on last day of therapy.

†Represents Wilcoxon signed rank test *P* values (adjusted). Values are compared to baseline (starting) values and $P \leq 0.05$ is significant.

ginning and the end of the study (table 2, fig 2). The percent of myeloblasts in the marrow rose from a beginning median value of 10% (range, 3%–20%) to a significantly ($P < 0.0136$) elevated ending median value of 15% (range, 4%–70%). Serial bone marrow chromosomal analysis was performed on seven of the patients. Five of the seven patients had nonrandom chromosomal changes, and these chromosomal abnormalities were present in all metaphases of the marrow cells examined, both at the initiation and completion of the therapeutic trial.

One patient (No. 4) had a 50% improvement in absolute concentration of granulocytes, monocytes, and platelets in her blood for > 5 sequential weeks of treatment (partial response, table 1). This patient, however, developed AML on Week 11 of therapy with $1,25(OH)_2D_3$. Seven patients had an improvement of either their absolute granulocyte, monocyte, or platelet blood concentrations for > 4 sequential weeks of treatment (minor response, table 1). However, one of these patients (No. 2) developed AML while receiving $1,25(OH)_2D_3$. Patient 12 died of hemorrhage with severe thrombocytopenia on the eighth week of $1,25(OH)_2D_3$ therapy. Three patients had no change in their granulocyte, monocyte, or platelet counts. By the conclusion of the therapeutic trial, seven patients had progressed to AML with > 10% blast cells in the peripheral blood and > 30% blasts in the bone marrow (table 1). Six of these patients received combination chemotherapy, and the other patient died without therapy.

Toxicity of $1,25(OH)_2D_3$ in Myelodysplastic Patients (Preleukemia)

The serum calcium increased in each of the patients while they were receiving $1,25(OH)_2D_3$. The serum calcium was a median of 8.95 mg/dl (patient range, 8.2–10.4; normal volunteer range, 8.8–10.6) prior to therapy, a peak median of 10.8 mg/dl (range, 9.7–17) during therapy, and a median of 10.30 mg/dl (range, 9.6–12.4) at the end of the study (table 2, fig 2). The peak serum calcium of nine of 18 patients was > 11 mg/dl. Four of these patients had one or all of the following symptoms: polydypsia, polyuria, nausea, lethargy, and confusion. Patient 6 developed a serum calcium of 17.0 mg/dl and became markedly confused. Three of the four patients with symptoms had independently increased their intake of $1,25(OH)_2D_3$ to ≥ 4.0 μg/day. The $1,25(OH)_2D_3$ was temporarily discontinued in each of the symptomatic hypercalcemic patients, and all patients recovered within 1–2 days without hospitalization. The $1,25(OH)_2D_3$ was restarted at a 0.5-μg/day lower dose. The patients had no other toxic effects associated with the administration of $1,25(OH)_2D_3$.

DISCUSSION

The seco-steroid $1,25(OH)_2D_3$ is capable of inducing differentiation of promyelocytic HL-60 cells (6,12–15). Concentrations as low as 5×10^{-9} M $1,25(OH)_2D_3$ induce dif-

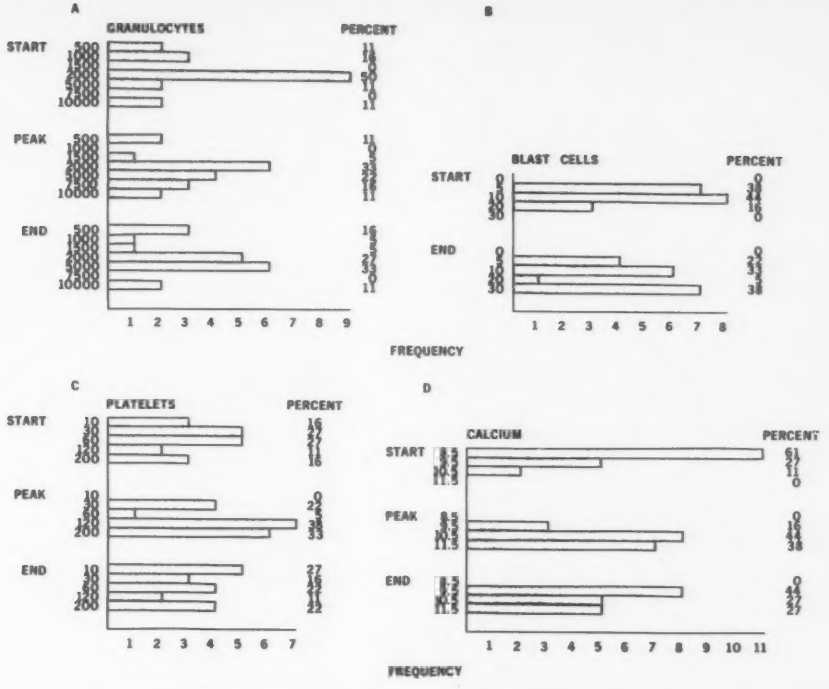

FIGURE 2.—Effect of administration of $1,25(OH)_2D_3$ on hematopoiesis in myelodysplastic (preleukemic) patients. A: effect of $1,25(OH)_2D_3$ on peripheral blood granulocyte concentrations (per μl of blood); B: effect of $1,25(OH)_2D_3$ on % of bone marrow myeloblasts; C: effect of $1,25(OH)_2D_3$ on peripheral blood platelet concentrations (per μl of blood); and D: effect of $1,25(OH)_2D_3$ on serum calcium concentrations (mg/dl); normal range of serum calcium is 8.2–10.6 mg/dl. Start = baseline value; peak = highest value during study; and end = value on last day of study.

ferentiation of approximately 50% of the cells as judged by morphology, cytochemistry, and function. The mechanism of $1,25(OH)_2D_3$ induction of differentiation of HL-60 is not known. The HL-60 cells contain about 4000 high-affinity (Kd $\sim 2.3 \times 10^{-9}$ M) $1,25(OH)_2D_3$ receptor molecules per cell (15). The concentration of $1,25(OH)_2D_3$ (2.3×10^{-9} M) which facilitates the 50% saturation of receptors in HL-60 cells is nearly identical to the concentration of this sterol that induces 50% of the cells to undergo differentiation (15). Likewise, studies showed that the potency of induction of differentiation of HL-60 by different vitamin D metabolites is directly correlated with the potency of binding of the metabolite to the $1,25(OH)_2D_3$ receptors (12). Taken together, these findings suggest that $1,25(OH)_2D_3$ induces differentiation of hematopoietic cells by binding of the steroid to cellular $1,25(OH)_2D_3$ receptors; the $1,25(OH)_2D_3$-receptor complex presumably is bound to DNA and alters transcriptional control.

Interest has developed in attempting to induce differentiation of myeloid leukemic blast cells in patients to functional, mature cells that are no longer capable of proliferation (6–10). Such an approach might eliminate or decrease the amount of cytotoxic chemotherapy needed for certain patients with leukemia and improve the hematopoiesis of preleukemic patients.

We show that a subpopulation of leukemic blast cells taken from patients can be induced to undergo differentiation in vitro by 10^{-6} to 10^{-7} M $1,25(OH)_2D_3$. In contrast, 10^{-9} M $1,25(OH)_2D_3$ had little effect on differentiation. In the flasks containing 10^{-6} M $1,25(OH)_2D_3$, approximately 40% (median) of leukemic cells differentiated as monitored by morphology and development of the ability to produce superoxide (NBT reduction), and about 15% (median) of the blast cells developed the ability to phagocytose *Candida*. In another series of experiments, we showed that $1,25(OH)_2D_3$ can induce differentiation of leukemic myeloid colony-forming cells to form colonies containing mature myeloid cells (24). Concentrations of 10^{-8} M of the sterol induced about 50% of myeloid leukemic colony-forming cells to form colonies composed of differentiated cells. In contrast, only 10%–15%

of leukemic myeloid colony-forming cells formed colonies of mature cells in agar containing no $1,25(OH)_2D_3$.

Human myeloid leukemia is a heterogenous group of diseases with different morphologies, karyotypes, etc. Specific leukemia subtypes (eg, acute promyelocytic leukemia) may have cells amendable to induction of terminal differentiation. We found that the greatest maturation that was induced by $1,25(OH)_2D_3$ occurred in flasks containing blast cells of the more mature myeloid stages of development. Likewise, we and others previously showed that the $1,25(OH)_2D_3$ (10^{-6} to 10^{-10} M) can induce differentiation of cells from human myeloid lines blocked at mature stages of differentiation (eg, HL-60 promyeloblasts, U937 monoblasts, THP-1 monoblasts), but the same concentrations of the steroid cannot induce differentiation of the relatively immature myeloblasts (HL-60 blast, KG-1a, KG-1) and the bipotent erythromyeloid blast cells (K562) (6,15). Taken together, the findings suggest that $1,25(OH)_2D_3$ is a moderately potent inducer of differentiation of leukemic blast cells which are normally blocked at mature stages of maturation (M2-M4).

Preleukemic patients represent a particularly interesting group for testing of compounds that might induce differentiation. Data suggest that a population of neoplastic preleukemic cells can mature in vitro, and while preleukemic cell differentiation is less than normal, maturation is clearly greater than that seen in AML cells (1-4). No effective therapy exists for preleukemic patients at this time. The progression of their disease to AML is usually slow enough to allow a careful trial of a potential inducer of differentiation with appropriate monitoring for changes in hematopoietic parameters.

We attempted to improve the peripheral blood cytopenias and bone marrow-ineffective hematopoiesis in patients with preleukemia by having the patients ingest pharmacological doses of $1,25(OH)_2D_3$. The in vivo administration of $1,25(OH)_2D_3$ produced only a very temporal improvement in hematopoietic parameters in eight of the patients. No improvement of hematopoiesis was evident at the end of the study. The prognosis of preleukemic patients is quite variable; however, most patients with refractory anemia with excess blasts in transformation (RAEB-TR) or RAEB have an inexorable deterioration of hematopoiesis and often develop leukemia within months (25). We observed that seven patients with RAEB-TR or RAEB developed AML by the end of their trial of $1,25(OH)_2D_3$. It is impossible to know if the administration of $1,25(OH)_2D_3$ exacerbated their disease without a comparison to a comparable control group of preleukemic patients who received no therapy.

The preleukemic patients received at least 2 μg/day of $1,25(OH)_2D_3$. This dose results in serum concentrations of $1,25(OH)_2D_3$ of about 2.0×10^{-10} M in normal individuals (26). A concentration of 2×10^{-10} M $1,25(OH)_2D_3$ induces maturation of < 20% of HL-60 promyelocytes in vitro (6,12-15), and the present study shows that 10^{-9} M $1,25(OH)_2D_3$ has a negligible effect in vitro on the maturation of fresh leukemic blast cells.

Theoretically, the obtainment of higher serum concentrations of $1,25(OH)_2D_3$ in our preleukemic patients would have been desired. During the study, however, nine of 18 patients became hypercalcemic. Three patients who independently increased their dose of $1,25(OH)_2D_3$ to 4-6 μg/day became symptomatically hypercalcemic. Somewhat higher concentrations of $1,25(OH)_2D_3$ could have been given if the patients had been placed on a low-calcium diet. In the future, vitamin D analogs that induce hematopoietic cell differentiation without inducing hypercalcemia might be medically useful compounds for selected patients with preleukemia and leukemia. Likewise, combinations of inducers of differentiation may be more effective than any one agent. The induction of differentiation of myeloid leukemic cells to functional end cells offers an appealing, but unproven, therapeutic prospect.

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Enhancement of the Antitumor Efficacy of Lomustine by the Radiosensitizer RSU 1069[1,2]

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Previous investigations have shown that combining the radiation sensitizer misonidazole with conventional alkylating chemotherapeutic agents can lead to a therapeutic advantage. More recently, another sensitizer, RSU 1069, has been reported to give an enhancement of antitumor agent efficacy similar to that observed with misonidazole, but at an approximately tenfold lower sensitizer dose. One chemotherapeutic agent whose activity has been modified by sensitizers to a greater extent in tumors than in critical normal tissues is the nitrosourea lomustine (CCNU). The present studies evaluated the therapeutic benefit of combining RSU 1069 and CCNU in KHT sarcoma-bearing C3H/HeJ mice. The drugs were administered ip, and tumor response was assessed by measuring the survival of clonogenic KHT cells 22–24 hours after treatment. Normal tissue toxicity was determined using peripheral wbc counts 3 days after treatment and a 30-day lethality assay. Combining CCNU with a 0.38-mmol/kg dose of RSU 1069 increased tumor cell killing by a factor of ~ 1.9. Wbc toxicity and 30-day animal lethality increased with CCNU dose, but the addition of RSU 1069 enhanced either endpoint only slightly (factor of 1.0–1.2). The addition of RSU 1069 to CCNU treatment, therefore, led to a significant therapeutic benefit. [Cancer Treat Rep 69:1409–1414, 1985]

The potentiation of certain chemotherapeutic agents, particularly the alkylators lomustine (CCNU), cyclophosphamide (CYC), and melphalan (L-PAM), by nitroimidazole sensitizers has been well established (1–4). In a variety of model systems it has been shown that combining such anticancer agents with sensitizers like misonidazole (MISO) results not only in improved tumor responses but generally also in therapeutic benefits. Since the original observation that MISO could function not only as a radiosensitizer but also as a chemopotentiator, a number of investigations have been directed at evaluating other radiation sensitizers in combination with anticancer drugs (3–5). The majority of these have failed as chemopotentiators, and even those sensitizers which enhanced the tumoricidal effects of chemotherapeutic agents generally did not yield therapeutic benefits as large or larger than MISO. While there has been some in vivo evidence that nitroimidazoles more lipophilic than MISO are more effective as potentiators of the antitumor action of CCNU and L-PAM (6–9), such combinations also proved to be more toxic. In general, the use of nitroimidazoles more lipophilic than MISO along with these chemotherapeutic agents failed to provide an increase in therapeutic benefit compared to MISO (6,7). A similar conclusion also was reached when CYC was combined with sensitizers of varying lipophilicities (10,11).

Recently, there has been considerable interest in the radiosensitizing and chemopotentiating activity of the compound 1(2-nitro-1-imidazolyl)-3-aziridino-2-propanol (RSU 1069). This agent, which includes an aziridine group in a 2-nitroimidazole, has been observed to demonstrate greater radiosensitizing activity than MISO both in vitro and in vivo (12,13). In addition, it has been shown to be a potent enhancer of L-PAM activity in vivo (12–15).

The aim of the present investigations was to further characterize RSU 1069 as a chemopotentiator. In particular, experiments were designed to evaluate the ability of RSU 1069 to enhance the antitumor efficacy of CCNU and to determine whether such a combination could lead to a greater therapeutic gain than the combination of CCNU and MISO.

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MATERIALS AND METHODS

Animals and Tumors

In both the tumor and normal tissue toxicity studies, 8-14-week-old female C3H/HeJ mice obtained from Jackson Laboratories (Bar Harbor, ME) were used. KHT sarcoma cells (16) were prepared from solid tumors by mechanical dissociation (17) and maintained in vivo. For experiments, 2×10^5 tumor cells were inoculated im into the hind limbs. Once the tumors reached a size equivalent to a weight of $\sim$ 0.3-0.5 g, the mice were treated with the chemotherapeutic agent either alone or in combination with the sensitizer, or kept as untreated controls.

Drug Treatments

CCNU was kindly provided by Dr. Robert Engle of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. RSU 1069 was a gift from Drs. I. Stratford and I. Ahmed of the M. R. C. Laboratories, Harwell, England. CCNU was initially dissolved in 100% EtOH (10 mg in 1 ml) until just prior to injection, when 9 ml of a 0.3% solution of hydroxypropyl cellulose in sterile saline was added to the CCNU stock solution. RSU 1069 was dissolved in sterile saline at 8 mg/ml. All drug injections were ip on the basis of animal body weight.

Tumor Response

Response to treatment was assessed using an in vivo to in vitro tumor excision assay (7,18). KHT sarcoma cell survival was assayed 22-24 hours after treatment. The tumors were excised and single-cell suspensions prepared using a combined mechanical and enzymatic dissociation procedure (17,18). The cells were counted and various dilutions plated into 24-well plates with 10^4 lethally irradiated tumor cells in 0.2% agar containing alpha minimum essential medium supplemented with 10% fetal calf serum. In about 2 weeks, the surviving cells formed colonies which were counted with the aid of a dissecting microscope.

Normal Tissue Toxicity

Normal tissue toxicity was determined by measuring either peripheral wbcs 3 days after treatment, or the number of mice that died during a 30-day posttreatment period. Peripheral wbc counts were determined from 10-μl samples taken from the tail veins of female C3H/HeJ mice treated with a range of CCNU doses in the absence or presence of RSU 1069. Samples were taken 3 days after treatment, since at this time a nadir in the number of peripheral wbcs was seen with CCNU or CCNU plus sensitizers (19-21). The sample was diluted in 10 ml of saline and the rbcs lysed by adding a rbc-lysing agent. Blood cell counts were made on a Coulter Counter and Channelyzer System (Model C1000). Differential counts on each sample were made from blood smears that were air-dried and stained with Wright's Giemsa stain.

Whole animal toxicity for CCNU ± RSU 1069 was determined by recording the number of mice that died during a 30-day posttreatment period. From these data, the $LD_{50/30}$ values (doses lethal to 50% of the mice in 30 days) and 95% confidence intervals were calculated using a logit bioassay (22) and Scheffe's discrimination interval (23).

RESULTS

Figure 1 shows the effect on clonogenic cell survival in the KHT sarcoma of administering a 0.38-mmol/kg dose of RSU 1069 at various times before or after a 5.0-mg/kg dose of CCNU. The data indicate a clear enhancement of CCNU activity (cell survival is reduced by a factor of $\sim$ 5-10) when this sensitizer was given within 90 minutes (before or after) of the nitrosourea treatment. On the basis of the results illustrated in this figure, in all subsequent experiments RSU 1069 preceded CCNU administration by 30 minutes.

Clonogenic cell survival following a combination of a fixed dose of CCNU (5.0 mg/kg) and various doses of RSU 1069 is shown in figure 2. Over the range of RSU 1069 doses studied, clonogenic cell survival following the

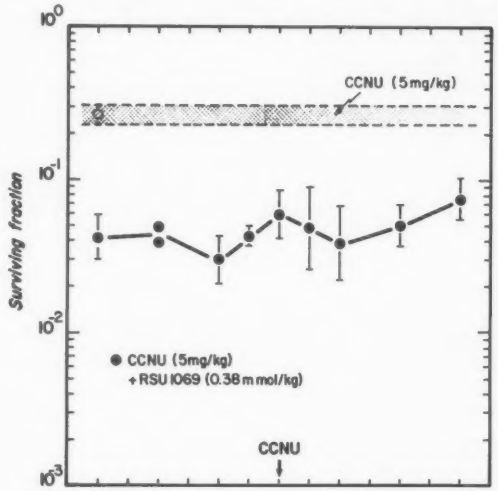

FIGURE 1.—Effect on KHT sarcoma cell survival of administering a 0.38-mmol/kg dose of RSU 1069 at various times prior to or following a 5.0-mg/kg dose of CCNU. Data shown are the mean ± SE of 3 experiments.

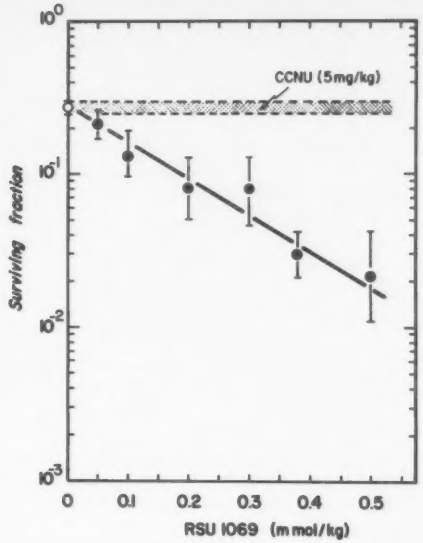

FIGURE 2.—Cell survival in KHT sarcomas treated with a 5.0-mg/kg dose of CCNU and a range of doses of RSU 1069. The sensitizer was administered 30 mins prior to the nitrosourea. The results are the mean of 3 experiments (± SE).

CCNU-sensitizer combination decreased exponentially with increasing sensitizer dose.

The effect of various doses of CCNU on tumor cell killing in the KHT sarcoma is illustrated in figure 3 (○). Also shown is the cell survival curve obtained when tumors are exposed to 0.38 mmol/kg of RSU 1069 30 minutes prior to CCNU treatment (●). While the sensitizer alone had little effect, the presence of RSU 1069 significantly enhanced tumor cell killing by CCNU. From the data, an enhancement ratio (ER), defined as the ratio of the dose of CCNU alone divided by the dose of CCNU plus RSU 1069 required to yield the same level of cell kill, was calculated. The results showed that RSU 1069 was dose-modifying when combined with CCNU and led to an ER of ~ 1.9.

In order to assess whether a combination of agents can lead to a therapeutic benefit, such a combination must be evaluated in terms of normal tissue toxicity. In these experiments, wbc counts were made on blood samples taken from the tails of C3H/HeJ mice. Previous investigations (19,21) had shown that CCNU and CCNU plus sensitizer exposures reduced wbc counts between 2 and 4 days after treatment, followed by recovery. Figure 4 shows total wbc counts 3 days after treatment, with a range of CCNU doses administered either alone (○) or in combination with a 0.38-mmol/kg dose of RSU 1069 (●). No consistent enhancement of wbc toxicity was seen when RSU 1069 was included in the CCNU treatment. Differential cell counts made on blood smears indicated that peripheral granulocytes were most affected by the CCNU ± RSU 1069 exposures. As seen in the total wbc counts for the various CCNU doses evaluated, the nadir in this cell population was not significantly altered by RSU 1069 (data not shown).

Systemic toxicity due to CCNU and CCNU plus RSU 1069 was also assessed. Previous investigations (21,24) have shown that following the administration of nitrosoureas either alone or in combination with sensitizers, most animals die from gut toxicity within 5–9 days. However, since CCNU is also toxic to the bone marrow (21,24,25), and since a few animal deaths occurred at times usually associated with bone marrow toxicity, the mice were observed for 30 days after treatment to include both the gut and bone marrow toxicity syndromes. Figure 5 shows the results of three experiments, which indicate that pretreating mice with 0.38 mmol/kg of RSU 1069 had only a relatively small effect on CCNU toxicity. $LD_{50/30}$ values determined from the experiments are shown in table 1. The data demonstrate that compared to CCNU alone, systemic toxicity in the CCNU plus RSU 1069 combination was enhanced 1.0–1.2-fold.

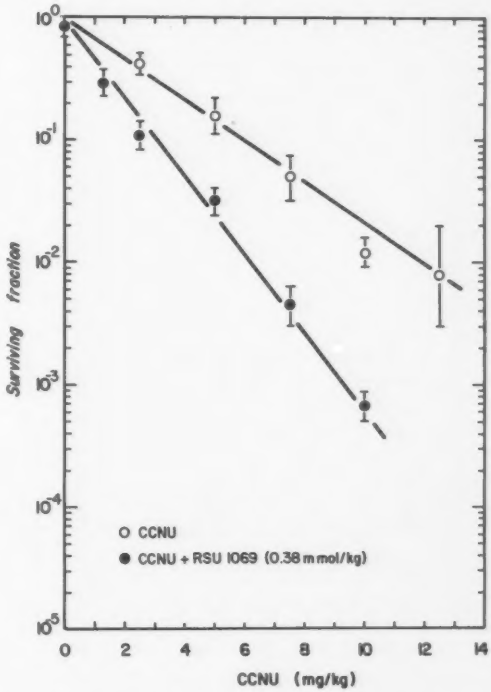

FIGURE 3.—Cell survival in KHT sarcomas treated with a range of CCNU doses either alone (○) or in combination with a 0.38-mmol/kg dose of RSU 1069 (●). RSU 1069 preceded CCNU by 30 mins. Data are the mean ± SE of 4 experiments.

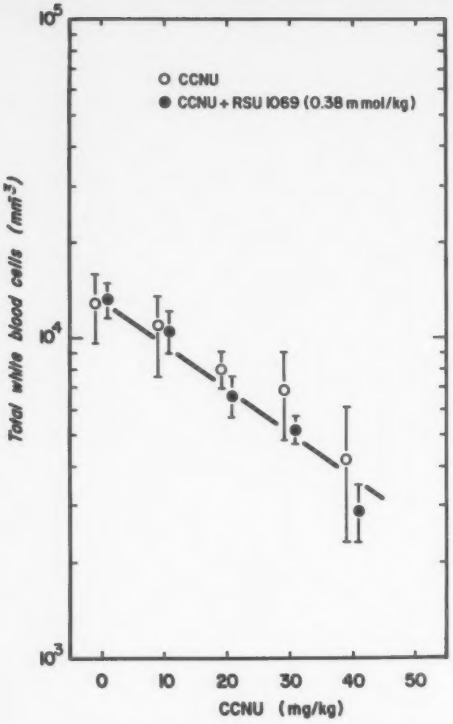

FIGURE 4.—Total wbc counts determined in female C3H/HeJ mice 3 days after exposure to a range of CCNU doses given either alone (○) or 30 mins following a 0.38-mmol/kg dose of RSU 1069 (●). Each data point is the mean ± SD on a sample of 5 mice.

DISCUSSION

The majority of animal tumor studies aimed at evaluating nitroimidazoles as potentiators of alkylating agents have indicated not only enhanced antitumor efficacies of agents such as CCNU, CYC, and L-PAM, but also that such combinations could lead to a therapeutic gain (1-4). In general, enhancement of critical normal tissues, such as the bone marrow or the gastrointestinal tract, was substantially less than the concomitant improvement in tumor response. The nitroimidazole most extensively studied in these investigations was MISO. Although the original animal investigations primarily utilized MISO doses too large to be attainable in man (2.5-5.0 mmols/kg), recent reports have demonstrated that at clinically achievable sensitizer doses, improved tumor responses could be attained in the absence of normal tissue toxicity (4,6,20,25,26). Consequently, there has been considerable clinical interest in combining alkylating chemotherapeutic agents and MISO, and a number of phase I/II trials using MISO in this capacity have been initiated (27-30).

Other nitroimidazoles have been compared to MISO in an attempt to select more effective chemopotentiators. In general, this approach has met with only limited success, although recently the sensitizer RSU 1069 has been shown to be as effective as MISO at potentiating the action of L-PAM, but at approximately tenfold lower sensitizer doses (12-15). However, a reduced sensitizer dose required to produce chemopotentiation does not in itself necessarily indicate a larger therapeutic gain. For example, it has been previously observed that the sensitizer benznidazole, which enhanced the action of CCNU at substantially lower doses than did MISO, led to significantly increased normal tissue damage and consequently no therapeutic benefit (6) or a gain equivalent to that obtained with larger MISO doses (7).

To evaluate RSU 1069 further, chemopotentiation of CCNU by this sensitizer was assessed in the present study in the KHT sarcoma as well as in terms of systemic host toxicity. The data in figure 3 show that the addition of a 0.38-mmol/kg dose of RSU 1069 enhanced the CCNU antitumor efficacy by a factor of ~ 1.9. This ER is similar to the value previously reported in the KHT sarcoma for CCNU combined with 5.0 mmol/kg of MISO (1,3). Consequently, these clonogenic tumor cell survival studies confirm previous results with L-PAM, which indicated that RSU 1069 was more effective as a chemopotentiator than MISO on an administered dose basis (12-15).

A number of mechanisms are possible for the enhancement of the antitumor efficacy of alkylating chemotherapeutic agents by nitroimidazoles. These have been discussed in detail elsewhere (1-5). Briefly, they include altered chemotherapeutic agent pharmacokinetics, enhanced DNA damage/reduced DNA damage repair, or intracellular thiol depletion. The present data provide no direct evidence for the mechanism(s) responsible for the enhanced tumor cell killing seen when RSU 1069 is included in a CCNU treatment. However, results showing that the addition of RSU 1069 90 minutes *after* CCNU administration can lead to significant chemopotentiation (fig 1) are difficult to reconcile with a pharmacologic mechanism (4).

TABLE 1.—Systemic toxicity of CCNU or CCNU + 0.38 mmol/kg of RSU 1069 in female C3H/HeJ mice

Experiment	$LD_{50/30}$* (mg/kg)		ER†
	CCNU	CCNU + RSU 1069	
a	39.8 (37.8-41.8)	40.3 (38.3-42.3)	1.0
b	44.6 (43.9-45.2)	37.5 (36.8-38.2)	1.2
c	42.5 (41.3-43.7)	40.0 (38.9-41.2)	1.1

*$LD_{50/30}$: drug dose (95% confidence interval) yielding 50% animal lethality in 30 days, calculated using a logit bioassay (ref 22) and Scheffe's discrimination interval (ref 23).

†Dose of CCNU alone divided by dose of CCNU + RSU 1069 required to yield 50% animal lethality.

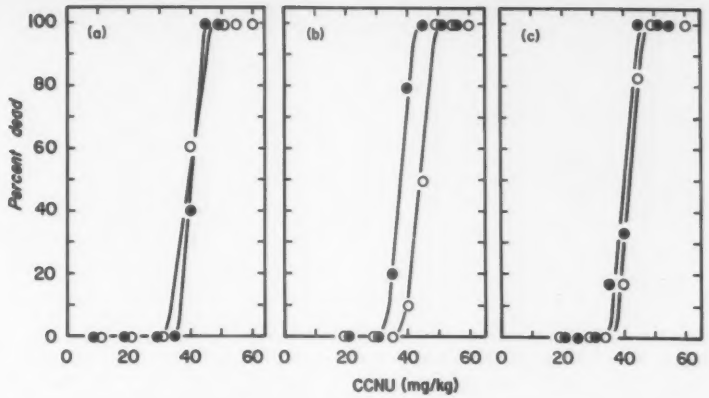

FIGURE 5.—Effect of pretreatment with a 0.38-mmol/kg dose of RSU 1069 (●) on the 30-day survival of non-tumor-bearing C3H/HeJ mice treated with CCNU. Open symbols are the results for mice receiving CCNU without the sensitizer. Three separate experiments are shown. Each data point represents 5–12 mice.

Combining a 0.38-mmol/kg dose of RSU 1069 with CCNU did not increase the peripheral wbc toxicity of this chemotherapeutic agent (fig 4; ○ vs ●). However, the systemic toxicity of the CCNU plus RSU 1069 combination, determined using the $LD_{50/30}$ as an endpoint, was greater (by a factor of 1.0–1.2) than that observed for CCNU alone (fig 5, table 1). Previous investigations, evaluating primarily bone marrow and gastrointestinal toxicity, have shown that a 5.0-mmol/kg dose of MISO enhanced the toxic effects of CCNU in these tissues by a factor of 1.0–1.4 (1,3). Long-term effects of CCNU alone or in combination with radiosensitizers are currently under investigation in our laboratories. Preliminary investigations focusing on lung toxicity have failed to demonstrate significant pulmonary damage in those mice surviving the gut and bone marrow syndromes (Siemann and Macler, unpublished data).

The present results indicate that the combination of CCNU plus RSU 1069 yields a therapeutic benefit. The gain achieved by combining a 0.38-mmol/kg dose of this sensitizer with CCNU is at least as large as, if not larger than, that previously observed when a 5.0-mmol/kg dose of MISO and CCNU were combined. The present results suggest that RSU 1069 be further evaluated as a chemopotentiator.

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Synthesis, Toxicity, and Therapeutic Efficacy of 4-Amino-*N*-(2′-aminophenyl)-benzamide: A New Compound Preferentially Active in Slowly Growing Tumors[1]

Martin R. Berger,[2,*] Helge Bischoff,[2] Edgar Fritschi,[3] Thomas Henne,[2] Manfred Herrmann,[3] Beatrice-Louise Pool,[2] Gerhard Satzinger,[3] Dietrich Schmähl,[2] and Ute Weiershausen[3]

The present paper describes 4-amino-*N*-(2′-aminophenyl)benzamide (GOE1734) with regard to synthesis; toxicity in mice, rats, and dogs; and differential therapeutic efficacy in slowly and rapidly proliferating rat tumors. GOE1734, an analog of a group of compounds known for other than antitumor effects with relatively simple *N*-acyl-*O*-phenylenediamine structure, is characterized by a low bacterial mutagenic potential after in vitro metabolic activation and DNA-DNA crosslinking activity after in vivo treatment. Maximum tolerated doses in rats and dogs amount to 4 and 1 mg/kg, respectively. High growth-inhibiting efficacy was obtained in intratibially implanted osteosarcoma, in methylnitrosourea-induced primary mammary carcinoma, and in acetoxymethyl-methylnitrosamine-induced colorectal adenocarcinoma. GOE1734 proved to be ineffective in transplanted Yoshida sarcoma and Walker 256 carcinosarcoma when single or multiple doses were administered at dose levels that were moderately toxic or not toxic. Some antitumor effects were observed in L5222 leukemia after ip transplantation, but no effect could be observed after ic implantation or in vitro incubation and subsequent retransplantation of these cells. Since the latter three rat tumors are characterized by relatively short tumor volume doubling times (0.5–2 days), whereas the first three grow slower (tumor volume doubling time, 11–19 days), the remarkable differential antitumor activity of GOE1734 in fast and slowly growing malignancies is striking. [Cancer Treat Rep 69:1415–1424, 1985]

Currently used cytostatic drugs are able to achieve normal lifespans only in patients with certain types of cancer (1–3). The responsive category does not include the most frequent forms of malignant tumors, but it includes neoplasms with relatively short tumor volume doubling times (3,4). This selection may be a consequence of former screening programs, which were based on serially transplanted tumors (5,6). These are believed to preferentially select drugs active against rapidly proliferating malignancies (3). As a consequence, attention has been focused on the utilization of slowly growing tumors for drug selection and evaluation (3,6). In addition to human tumor xenografts, chemically induced autochthonous (primary) carcinomas are models which satisfy these new requirements with regard to growth kinetics (7) and chemosensitivity (8,9). They are therefore believed to be suitable models to predict therapeutic activity in man. Some clinically used drugs are active in rapidly growing tumors and to a lesser extent in slowly growing tumors (6). However, no compound has yet been evaluated in man that exerted high activity in the latter category only.

In this paper we report on synthesis, toxicity, and antitumor efficacy of 4-amino-*N*-(2′-aminophenyl)-benzamide (GOE1734) in fast and slowly proliferating tumor models. GOE1734 is an analog of a group of pharmacologically active lipophilic compounds with relatively simple *N*-acyl-*O*-phenylenediamine structure. Originally characterized as a potentially anticonvulsive agent, GOE1734 (fig 1) was suggested to have cytostatic properties since a marked inhibition of peripheral blood cells and of spermatogenesis was observed in rats after continuous application. The compound proved to be inactive against mouse leukemia P388 in the standard National

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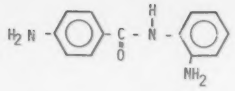

FIGURE 1.—Structure of GOE1734.

Cancer Institute screen but was found to be highly active in a preliminary study carried out by us against primary rat mammary carcinomas induced by methylnitrosourea (MNU). These results prompted us to evaluate GOE1734 in a broader test system consisting of four transplanted and two chemically induced autochthonous tumor models.

MATERIALS AND METHODS

Chemicals

GOE1734 was synthesized according to described methods (German Patent Application 3305 755 9) by condensation in dioxane of 4-nitrobenzoylchloride with 2-nitrobenzamine and hydrogenation with Raney-Ni in *N,N*-dimethylformamide (DMF) of the intermediate *N*-(2-nitrophenyl)-4-nitrobenzamide. GOE1734 ($C_{13}H_{13}N_3O$; molecular weight, 227.3) is a colorless, crystalline compound (melting point, 179°C in methanol) which is insoluble in water. The dihydrochloride forms up to 4% aqueous solutions (pH 2–3). For experimental purposes GOE1734 was suspended in 0.8% methocel (Nordmann-Rasmann, Hamburg, West Germany) at a concentration of 6 mg/ml.

MNU and acetoxymethyl-methylnitrosamine (AMMN) were synthesized by Dr. M. Wiessler (Institute of Toxicology and Chemotherapy, German Cancer Research Center); MNU was dissolved at 1% in Sörensen buffer (pH 6) and distilled water (20/80; vol/vol); AMMN was dissolved at 0.2% in physiological saline. Fresh solutions were prepared shortly before use.

Animals

Male Sprague-Dawley rats (Charles River Breeding, WiGa Sulzfeld, West Germany), male BD IX rats, and female Sprague-Dawley rats (Zentralinstitut für Versuchstierkunde, Hannover, West Germany) were kept under conventional conditions (temperature, 22°C ± 2°C; relative humidity, 55% ± 10%; dark-light sequence of 12 hours). Altromin pellets and tap water were given ad libitum. All animals were inspected twice daily and observed until their natural death except when indicated in a different manner. Dead animals were completely dissected to establish tumor and toxicity findings; macroscopically changed organs and autochthonous tumors were examined histologically.

Evaluation of Toxicity

GOE1734 was administered perorally by gavage at logarithmically spaced doses to groups of ten mice or rats each that had been fasted overnight. Acute toxicity was established on the basis of macroscopic necropsy findings in all dead animals (10,11).

Surviving animals were observed for 7 (mice) or 14 (rats) days. LD50 values were determined by the method of Spearman and Kärber (12). Additionally, prolonged peroral administration of GOE1734 in rats (12 weeks) and in dogs (14 weeks) was performed with weekly hematological examinations. At termination of these studies all animals were examined histologically, hematologically, and according to clinical chemistry parameters.

Mutagenicity

Bacterial mutagenicity was determined with the plate incorporation assay of Ames et al (13) using strains *Salmonella typhimurium*, TA 1538, TA 98, and TA 100 with and without metabolic activation (14).

Alkaline Elution Technique

DNA interstrand crosslinking and DNA single-strand breaks were measured in isolated liver cells as described by Kohn et al (15), with slight modifications (16,17).

Transplanted Tumors

All transplanted tumors were maintained under serial passage. For experimental purposes, 5×10^6 Yoshida sarcoma ascites cells were transplanted ip or sc to male Sprague-Dawley rats (18,19). Walker 256 carcinosarcoma ascites cells (1×10^6) were transplanted ip to female Sprague-Dawley rats according to described methods (19,20). Cells of acute rat leukemia L5222 were implanted ip [1×10^6 according to Zeller et al (21)] or ic [1×10^5 according to Fiebig et al (22)] to male BD IX rats. Fragments ($3 \times 1.5 \times 1.5$ mm) of osteosarcoma were transplanted intratibially to 11-day-old male Sprague-Dawley rats as previously described (23). In a combined in vitro-in vivo experiment, cells of the acute rat leukemia L5222, grown in culture, were incubated in a shaking water bath [RPMI-1640 medium enriched with 10% fetal calf serum (Gibco, Karlsruhe, West Germany)], L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μg/ml), and mercaptoethanol (5×10^5 M) over a period of 2 hours at 37°C together with rising concentrations of GOE1734. Thereafter, they were implanted ip (1×10^6 cells) to male BD IX rats.

Chemically Induced, Autochthonous Tumors

Autochthonous, hormone-dependent mammary carcinomas were induced by MNU in female Sprague-Dawley rats as previously described (24), with slight modifications (25). Rats with a total tumor volume of > 0.8 cm^3 were randomly allocated to experimental groups (Week 1, Day 1). Therapy was started immediately thereafter.

Induction of autochthonous colorectal adenocarcinomas with AMMN in male Sprague-Dawley rats was performed over 10 weeks, as previously described (26,27). Subsequent therapy of 10 weeks' duration was started 1 week after the end of induction. During therapy, tumor manifestation was followed with a children's bronchoscope (Olympus BF, 4 C 2, Olympus Optical Co, Tokyo). At the end of treatment all animals were killed. A colon section at a length of 20 cm proximal to the anus (containing all tumors induced) was resected and weighed separately. All tumors were excised and measured individually in three vertical axes. Tumor volumes were estimated according to the formula $a \times b \times c/2$.

Evaluation of Antitumor Activity

For ip implanted Yoshida sarcoma, Walker carcinosarcoma, and leukemia L5222 as well as for ic implanted leukemia L5222, the comparison of drug effectiveness was based on the increase in median survival time with the dose range used compared to untreated controls (T/C%).

For sc implanted Yoshida sarcoma, osteosarcoma, primary mammary carcinoma, and primary colorectal adenocarcinoma, tumor growth was assessed by caliper measurement, and tumor volume was calculated according to the formula $V = a \times b^2/2$ ($a > b$). Relation to percent untreated controls was used to estimate significance ($T/C\% \leq 42$).

RESULTS

Toxicity

Investigations of acute toxicity (table 1) showed higher LD50 values in mice (625 mg/kg) than in rats (95-212 mg/kg). The pattern of toxicity in both species was characterized mainly by sedation, ataxia, reduced muscle tone, and, at high doses, also by convulsions. At macroscopical examination the main findings were size reduction of spleen, liver, and lymph nodes; enteritis; petechial gastrointestinal bleedings; hemorrhagic cystitis; and hemorrhagic pulmonary edema.

Peroral daily administration of GOE1734 to rats over a period of 12 weeks at doses of 2, 4, and 6 mg/kg resulted in a significant, dose-dependent reduction in leukocyte and thrombocyte counts which were reversible on discontinuation of the substance. The decreases were coupled with a reduction in higher leukocytic maturity grades, appearance of damaged megakaryocytes, and early signs of loosening of erythropoietic islets in the bone marrow. At doses of ≥ 4 mg/kg, behavior, weight gain, and food intake were affected, and male and female gonads and the thymus glands showed involution. With escalating doses an increasing number of fatty deposits was found in the liver. These findings were much more pronounced in another investigation in rats with oral daily administration of 15, 30, and 45 mg/kg of GOE1734. All of the treated animals died, depending on the dose, within 6 weeks of this study. At these doses, the erythrocyte count was markedly decreased and myelopoietic and erythropoietic cell counts in bone marrow were almost totally reduced. Spleens, thymuses, and lymph nodes showed high-grade depletion of their lymphatic portions. All other findings were characterized by patterns of bacteria colonies due to agranulocytosis.

In dogs, GOE1734 was initially administered at daily doses of 1, 2, and 4 mg/kg orally. After 3 test weeks, the doses were increased to 2, 4, and 6 mg/kg, but had to be reduced again to the initial levels after 8 weeks. In the 1 (2) mg/kg dose group, there were no essential changes in findings compared to control animals. Dogs treated with 2 (4) and 4 (6) mg/kg presented retardation in body weight gain and dose-dependent reduction in all formed blood components which reversed when the dose was reduced or its application discontinued. Only in the high-dose group was a histologically visible decrease in megakaryocytes in the bone marrow observed, whereas the erythropoiesis and leukopoiesis were not influenced.

In experiments aimed at the detection of possible muta-

TABLE 1.—Acute peroral toxicity of GOE1734 in mice and rats

Species	Weight of animals (g)		LD50*	
	Median	Range	mg/kg	mg/m^2†
Mouse	18	17-20	625	1813
Rat	90	83-95	212	1060
Rat	140	124-157	190	1100
Rat	340	310-370	110	880
Rat	450	370-500	95	846

*According to the method of Spearman and Kärber (12).
†Conversion according to Freireich et al (10).

genicity of GOE1734, low mutagenic effects were seen in *Salmonella* strains TA 98 and TA 1538 (twofold to fourfold increase in spontaneous his$^+$ revertants up to 5000 μg of GOE1734/plate) only when a metabolizing mixture containing cofactors and 9000 × *g* supernatant of homogenized rat liver from aroclor pretreated Sprague-Dawley rats was present.

Sixteen hours after single oral doses of GOE1734 (10–156 mg/kg), considerable amounts of DNA single-strand breaks (124–294-rad equivalents) and DNA-DNA interstrand crosslinks (53–176-rad equivalents) were detected in rat liver cells by using the method of alkaline filter elution.

Carcinostatic Activity

Test results of GOE1734 against ip transplanted Yoshida sarcoma, Walker 256 carcinosarcoma, and acute rat leukemia L5222 are given in table 2. All three tumors were characterized by a median survival time of untreated controls between 8 (L5222) and 18 (Walker 256) days, depending on the respective number of cells implanted.

Peroral administration of logarithmically spaced dosages of GOE1734 using single- or multiple-application regimens did not prolong median survival times of animals bearing Yoshida or Walker 256 ascites. In contrast, total doses between 38 and 144 mg/kg, the latter dosage showing moderate toxicity, reduced the life expectancy of tumor-bearing animals.

Treatment of acute rat leukemia L5222 with GOE1734 proved to be somewhat effective. Oral doses of 48 or 38 mg/kg on Days 1, 4, and 7 and of 15 mg/kg on Days 1–9 after ip tumor implantation yielded T/C% values of 150 and 169, respectively. In a combined in vitro-in vivo experiment, L5222 cells were incubated for 2 hours at concentrations of 3, 30, and 300 μg/ml of GOE1734 and subsequently injected to rats that were observed until death. This treatment, however, did not result in significant prolongation of medial survival times (T/C%, 108 in all groups). A similar result was found in rats with ic

TABLE 2.—Antineoplastic activity in peroral application of GOE1734 in 3 ip transplanted rat tumors

Tumor*	Single dose (mg/kg)	Median survival time (range)†	T/C%	No. of toxic deaths/No. of rats treated
Yoshida sarcoma‡				
Experiment A	—	14.5 (9–51)	—	0/8
	75 (75)§	11.0 (8–14)	76	1/8
	60 (60)§	12.0 (9–13)	83	0/8
	48 (48)§	13.0 (10–19)	90	0/8
	38 (38)§	12.5 (12–61)	86	0/8
	24 (72)¶	11.5 (10–24)	79	0/8
	15 (45)¶	14.5 (9–26)	100	0/8
Experiment B	—	10.0 (9–12)	—	0/6
	48 (144)**	9.0 (7–11)	90	1/6
	38 (114)**	10.5 (9–15)	119	0/6
	24 (72)**	9.5 (8–15)	95	0/6
	15 (135)††	9.5 (7–11)	95	1/6
	10 (90)††	9.5 (8–11)	95	0/6
Walker 256 carcinosarcoma‡‡	—	18.0 (13–18)	—	0/6
	60 (60)§§	14.0 (11–18)	78	0/6
	48 (48)§§	14.0 (13–18)	78	0/6
	38 (38)§§	16.0 (12–17)	89	0/6
	24 (72)**	17.0 (14–18)	94	0/6
	15 (45)**	18.0 (13–18)	100	0/6
Acute leukemia L5222‡‡	—	8.0 (8–11)	—	0/6
	60 (180)**	10.5 (7–14)	131	1/6
	48 (144)**	12.0 (10–16)	150	0/6
	38 (114)**	12.0 (11–13)	150	0/6
	24 (216)††	9.0 (8–11)	113	1/6
	15 (135)††	13.5 (6–14)	169	0/6

*Day of tumor implantation = Day 0.
†Days from implantation of tumor.
‡Rats received 5 × 10^6 cells ip.
§Treatment on Day 5.
¶Treatment on Days 5, 8, and 11.
**Treatment on Days 1, 4, and 7.
††Treatment on Days 1–9.
‡‡Rats received 1 × 10^6 cells ip.
§§Treatment on Day 1.

inoculated leukemia (table 3). Although doses and regimens were applied that had been proven to have some effect in ip implanted tumors, there was no prolongation of survival times compared to untreated controls with respect to T/C%. Treatment of sc implanted Yoshida sarcoma was also ineffective (table 3). Significant inhibition of tumor growth with a T/C% value of < 42 was not reached in any of the cases. However, the lowest dose applied yielded the best therapeutic effect (T/C% = 52).

Therapy of intratibially implanted osteosarcoma resulted in 64% tumor growth retardation of this relatively slowly growing tumor (mean tumor volume doubling time of untreated controls, 11.5 days) (table 3). After 3 weeks of treatment, nine of ten treated animals were still alive, whereas seven of ten control animals had already died due to lung metastases, thus indicating an antimetastatic effect of this compound.

Experimental application of five dosages of GOE1734 to rats bearing MNU-induced mammary carcinoma is shown in table 4. Over a period of 4 weeks, the median tumor volume of untreated controls increased logarithmically and plateaued thereafter (Group I, fig 2). The mean tumor volume doubling time in these animals amounted to 11 days (95% confidence limits, 9–13 days). Administration of GOE1734 (2.5–12.5 mg/kg/day) both retarded the growth rate of developed mammary carcinomas and prevented the occurrence of new lesions in a dose-dependent manner. No significant antitumor activity was seen when doses of 2.5 mg/kg/day (Group II) were administered. Some efficacy could be obtained when this starting dose was doubled (Group III). The optimal dose amounted to 7.5 mg/kg/day, since this dose virtually arrested tumor growth and inhibited the manifestation of new tumors, although a concomitant mortality of 10% was observed (Group IV). Higher doses (10 mg/kg/day, Group V, and 12.5 mg/kg/day, Group VI) could not further increase the therapeutic effectiveness of GOE1734, but displayed considerable toxicity (20% and 80%, respectively) after a period of 5 weeks. Interestingly, the highest dosage (Group VI) was less effective in inhibiting tumor growth than the lower doses of Groups IV and V. A steep dose-response curve is indicated by these findings. T/C% values of the whole experiment are shown in figure 3.

After 5 weeks of treatment, application of GOE1734 was stopped in Group IV (daily dose, 7.5 mg/kg) for a period of 2 weeks, but continued in Groups III and V (daily dose, 5 and 10 mg/kg, respectively). As shown in figure 2, a sharp onset of tumor growth could be observed after the therapy was discontinued; this resulted in a tumor growth curve parallel to that of controls 5 weeks earlier. When the therapy was resumed at Week 8, tumor

TABLE 3.—Antineoplastic activity of perorally administered GOE1734 in 3 rat tumors transplanted to different sites

Tumor	Tumor inoculation	Single dose (mg/kg)	T/C%	No. of toxic deaths/No. of rats treated
Yoshida sarcoma*	Sc	—	100†	0/8
	Sc	60 (180)‡	150	6/8
	Sc	48 (144)‡	200	2/8
	Sc	38 (114)‡	62	0/8
	Sc	15 (135)§	76	4/8
	Sc	12 (108)§	76	2/8
	Sc	9 (81)§	52	0/8
Acute leukemia L5222¶	Ic	—	100	0/12
	Ic	48 (144)**	104	2/10
	Ic	24 (72)**	104	0/6
	Ic	12 (36)**	96	0/6
	Ic	24 (216)††	63	6/6
	Ic	15 (135)††	115	4/12
	Ic	10 (90)††	107	0/6
Osteosarcoma‡‡	Intratibially	—	100§§	0/10
	Intratibially	10 (150)¶¶	36	0/10
	Intratibially	5 (75)¶¶	96	0/10

* Rats received 5×10^6 cells.
† Evaluation on Day 21.
‡ Treatment on Days 6, 13, and 20.
§ Treatment on Days 6–15.
¶ Rats received 1×10^5 cells.
** Treatment on Days 1, 4, and 7.
†† Treatment on Days 1–9.
‡‡ Treatment started at median tumor diameter of 1.2 cm (range, 1.05–1.55).
§§ Evaluation on Day 22 after start of treatment.
¶¶ Treatment on Days 1–5, 8–12, and 15–19.

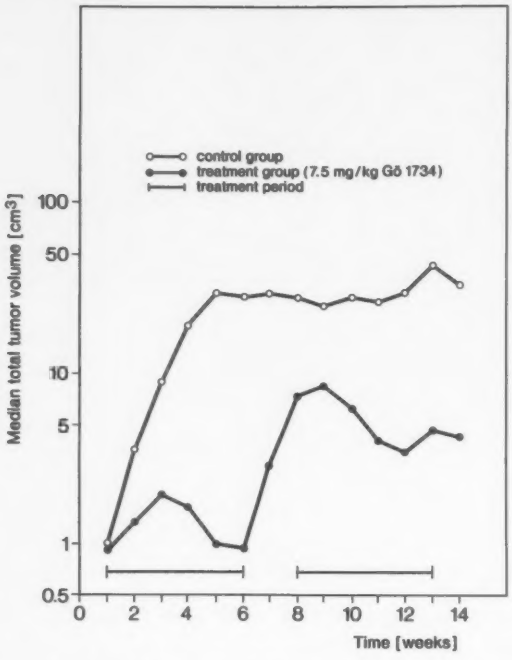

FIGURE 2.—Effect of administration, discontinuation, and resumption of GOE1734 on median total tumor volume of Sprague-Dawley rats bearing MNU-induced mammary carcinomas.

growth was retarded at once and decreased subsequently. Because of the continuation of therapy in Groups III and V, tumor growth remained constant in these groups without such an "escape phenomenon," thus indicating no signs of (possible) resistance induced by this therapy within a period of 10 weeks.

The antineoplastic efficacy of GOE1734 against AMMN-induced colorectal adenocarcinoma was tested in two subsequent experiments according to the design given in table 5. These tumors are characterized by a relatively long tumor volume doubling time of 18.5 days (95% confidence limits, 15.7-22.5 days). After 10 weeks of treatment, a dose-related survival rate was obtained, with 31% mortality in animals receiving the highest daily dose (10 mg/kg; Group VI) and no compound-related mortality in animals that received 5 mg/kg/day only (Group II). Under the highest dosage a positive weight difference of treated animals could be observed, thus indicating a good tolerance of the long-term treatment. Results of therapy, as measured by the median total tumor volume per rat and the median tumor number per rat, are given in figures 4 and 5, respectively. There is a dose-dependent decrease in median tumor volumes and tumor numbers with increasing dose, thus effecting significant T/C% values of 29 (Group IV) and 12 (Group VI) at a daily dose of 7.9 and 10 mg/kg, respectively. However, this latter dose was toxic as seen by both a high weight loss and a 31% mortality rate. Therefore, the corresponding active T/C value is not meaningful. The number of rats without tumors at the end of the treatment in-

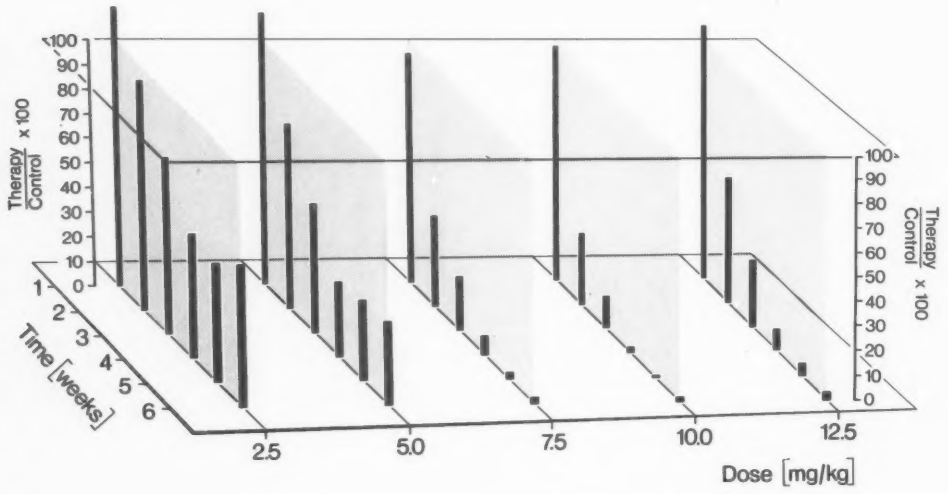

FIGURE 3.—Treatment of Sprague-Dawley rats bearing MNU-induced mammary carcinomas with GOE1734: mean tumor volume as % of untreated control animals in relation to dose and time.

TABLE 4.—Antineoplastic efficacy of perorally administered GOE1734 in MNU-induced mammary carcinomas of Sprague-Dawley rats

Group No.*	Median total dose (mg/kg)	Median tumor volume in cm^3 (95% confidence limits)			No. of tumors†	% body weight difference (Week 6–Week 1)	No. with mortality at Week 6 (%)
		Week 1	Week 3	Week 6			
I	—	1.0 (0.8–1.5)	9.1 (5.2–19.3)	28.0 (25.6–46.5)	4.7 ± 2.8	+13.6	0 (0)
II	62.5‡	1.1 (0.8–2.1)	6.4 (2.4–14.2)	16.7 (7.9–31.0)	3.5 ± 1.3	+14.6	0 (0)
III	125§	1.1 (0.8–1.7)	4.8 (3.2–10.8)	9.8 (0.8–14.4)	3.4 ± 1.5	+12.8	1 (10)
IV	187.5¶	0.9 (0.7–2.4)	2.0 (0.9–7.7)	1.0 (0.5–5.5)	1.0 ± 0.8	−4.2	1 (10)
V	250**	1.0 (0.8–3.1)	1.2 (0.4–4.4)	0.6 (0.1–4.3)	1.7 ± 1.1	−1.1	2 (20)
VI	187.5††	1.0 (0.8–3.2)	2.5 (0.3–5.9)	1.0 —	1.0 ± 1.4	−28.3	8 (80)

* Group I consisted of 20 rats, Groups II–VI of 10 rats each.
† Mean No. of tumors/rat developing newly under therapy ± SD.
‡ 2.5 mg/kg on Days 1–5/wk × 5.
§ 5 mg/kg on Days 1–5/wk × 5.
¶ 7.5 mg/kg on Days 1–5/wk × 5.
** 10 mg/kg on Days 1–5/wk × 5.
†† 12.5 mg/kg on Days 1–5/wk × 5.

creased from 3% (controls) to 64% in the highest dosage group. To discern local toxicity, the weight of distal colorectum of treated and untreated animals was determined. Subtraction of the median total tumor volumes ($mm^3 \approx mg$) from the respective median weight of distal colorectum reveals about the same values with the exception of the highest dosage group, which shows a considerably lower weight of distal colorectum, thus reinforcing the impression of general toxicity of this dosage.

The correlation between individual total tumor volumes and respective distal colorectum weights shows a significant ($\alpha = 0.01$) linear regression curve with $\gamma = 0.58$ in control animals, but indicates a nonsignificant relationship between both parameters in successfully treated animals.

DISCUSSION

The results presented in this report give evidence of toxicity in three species, mutagenicity in bacteria, mechanisms of toxicity in rat liver, and, in addition, differential therapeutic efficacy of GOE1734 in various experimental tumor models. Short-term toxicity studies substantiate nearly a twofold difference in tolerance between mice and rats. In addition, an increasing sensitivity of animals with higher body weights was observed. This property is not uncommon for cytostatic drugs (10,11). Long-term studies have revealed that the hematopoietic system, gonads, and liver are targets of specific organ toxicity. Cumulation of toxicity occurs within 2 weeks after first administration, a dose of 4 mg/kg being the maximum tolerated dose in rats and 1 mg/kg being the maximum tolerated dose in dogs.

Peroral treatment of ip implanted Yoshida sarcoma and Walker 256 carcinosarcoma with GOE1734 had no significant tumor-inhibitory effect. Both tumors have been transplanted serially for a considerable amount of time (18,28) and now have tumor volume doubling times of 0.5–1 day (4,7). A somewhat different observation was made after sc implantation of Yoshida sarcoma cells. These tumor cells grow at a slower rate when transplanted sc and an enhanced, although still insignificant, antitumor activity was observed. Similarly, ip implanted L5222, a leukemia with tumor volume doubling time of 0.5 day (29), was influenced in a marginally significant manner. This corresponds to results obtained in L1210 leukemia (30), which has the same tumor volume doubling time as L5222 (5). In this mouse leukemia only slight and barely significant activity could be found at one dose level. No activity was found after ic implantation of L5222 leukemia. Since there was also no activity against mouse leukemia P388 in the standard National Cancer Institute screen, it might be concluded that GOE1734 is not or at best is marginally active against a variety of quickly growing transplanted tumors in mice and rats. In contrast to these observations, a striking activity was

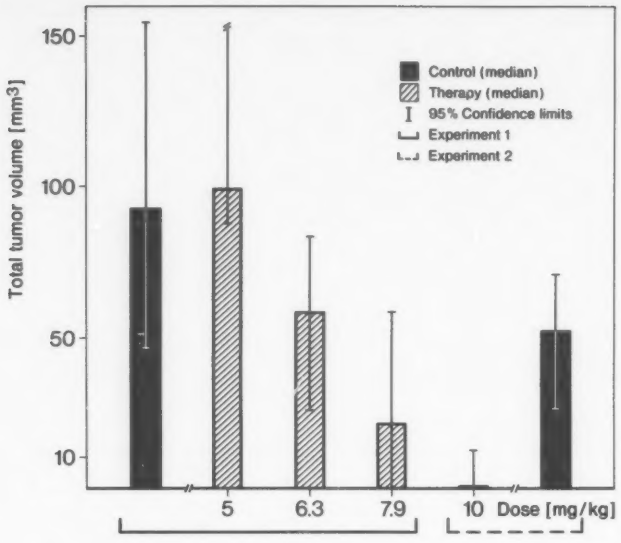

FIGURE 4.—Efficacy of GOE1734 on median total tumor volume in male Sprague-Dawley rats bearing AMMN-induced colorectal adenocarcinomas.

found in slowly growing rat tumors such as MNU-induced autochthonous mammary carcinoma, AMMN-induced colorectal carcinoma, and to a lesser extent in transplanted osteosarcoma. These findings are in accordance with results of Lelieveld et al (30), who obtained some growth delay (3–6 days) in sc implanted Lewis lung carcinoma, which is characterized by a volume doubling time of 2.9 days (5). They also correspond to results in increased lifespans of rats bearing BN myeloid leukemia cells (maximum T/C%, 430), a leukemia with tumor vol-

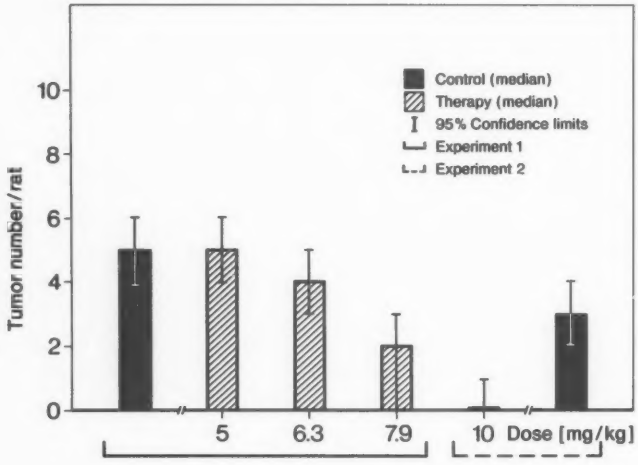

FIGURE 5.—Efficacy of GOE1734 on median No. of tumors in male Sprague-Dawley rats bearing AMMN-induced colorectal adenocarcinomas.

TABLE 5.—Antineoplastic efficacy of perorally administered GOE1734 in AMMN-induced colorectal adenocarcinoma of male Sprague-Dawley rats

Group No.*	No. of animals	Median total dose (mg/kg)	T/C%	No. of rats without tumors (%)†	Median weight of distal colorectum (95% confidence limits)‡	% body weight difference (Week 11-Week 1)	No. with mortality (%)†
I	36	—	100	1 (3)	2.55 (2.19-2.74)	+13.8	0 (0)
II	19	250§	107	0 (0)	2.49 (2.15-3.3)	+6.0	0 (0)
III	19	315¶	50	1 (6)	2.14 (1.88-2.33)	+5.7	1 (5.3)
IV	20	395**	29	6 (33)	1.91 (1.55-2.22)	+4.4	2 (10)
V	25	—	100	1 (4)	2.46 (2.28-2.85)	+8.0	0 (0)
VI	16	500††	12	7 (64)	1.69 (1.41-1.85)	−15.6	5 (31)

*Groups I-IV, Experiment 1; Groups V and VI, Experiment 2.
†Evaluation at Week 11.
‡Section of colorectum 20 cm proximal to the anus was weighed.
§5 mg/kg on Days 1-5/wk × 10.
¶63 mg/kl on Days 1-5/wk × 10.
**7.9 mg/kg on Days 1-5/wk × 10.
††10 mg/kg on Days 1-5/wk × 10.

ume doubling time of 2-10 days (31).

It should be stressed that GOE1734 was found to be especially active in both autochthonous models and was superior to all other compounds previously tested against these tumors, such as cyclophosphamide, nitrosoureas, doxorubicin, vincristine, methotrexate, 5-FU, etc (25-27,32,33).

The up-to-date mechanism of action of GOE1734 is unclear. Since there is some substructural similarity with folic acid, a folic acid antagonism seemed possible. A reversal of GOE1734-induced prolongation of rat skin allograft survival, however, was only partial when folinic acid was administered to animals simultaneously (Weiershausen, et al, manuscript in preparation). Nevertheless, antimetabolite activity is suggested since continuous application is needed for effective treatment. Furthermore, similar to other antimetabolites, the compound is carcinostatic rather than tumoricidal and there is a time lag until optimal inhibition of tumor growth is obtained (fig 2).

Low mutagenic activity was found after metabolic activation only. Positive *Salmonella* strains are not typically reverted by simple alkylating agents, but by frameshift-inducing agents (13). DNA single-strand breaks and DNA-DNA interstrand crosslinks were detected in liver cells of treated rats by the method of alkaline filter elution. Since in vitro incubation of L5222 cells with GOE1734 effected no increase in lifespan after retransplantation, it might be assumed that GOE1734 has to be activated, presumably in the liver, before interaction with DNA is possible. This interaction obviously leads to alkali labile sites within DNA as well as alkali stabile connections of DNA fragments, thus implying a bifunctionally crosslinking metabolite of GOE1734. *N*-Hydroxylation of both amino groups may be a first step during the generation of this active metabolite. A further detailed study on the genotoxic properties of GOE1734 in vitro and in vivo will be published separately. As discussed previously, additional activity as an antimetabolite cannot be excluded. In contrast, a hormonal mechanism seems to be improbable, although optimal tumoristatic efficacy was obtained in hormone-dependent MNU-induced mammary carcinoma.

In conclusion, GOE1734 is a new compound with high activity, preferentially in slowly growing tumors, and only marginal efficacy in rapidly proliferating systems. Further experiments, mainly on the mechanisms of action, are needed. With respect to metabolic activation which is indicated by mutagenicity studies and in vitro incubation experiments, one may expect species differences in the action of this compound. Clinical evaluation should clarify the possible advantage of this compound in the treatment of slowly growing human malignancies.

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Intensive Short-term Chemotherapy for Patients With Acute Myelogenous Leukemia: Long-term Follow-up[1]

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Our pilot study addresses the problem of early relapse from complete remission in young adults with acute myelogenous leukemia (AML). Twelve patients with AML, 16–58 years of age, were entered in a study of four intense courses of cytotoxic chemotherapy using the following drugs: cytarabine, daunorubicin, 5-azacitidine, and 6-thioguanine. They received no maintenance therapy. Nine of 12 patients achieved complete response. With a minimum follow-up of 35 months, the observed disease-free survival at 2 years was 67% (14 ± SE) and the actuarial disease-free survival at 4 years was 38% (17 ± SE). It appears that brief intensive chemotherapy early in the management of AML can produce prolonged remission without the need for maintenance therapy. [Cancer Treat Rep 69:1425–1427, 1985]

Recently published remission induction rates for acute myelogenous leukemia (AML) are 60%–80%. Unfortunately, the median duration of complete remission (CR) is frequently < 1 year (1–3). Intensive chemoradiotherapy of short duration followed by bone marrow transplantation during remission has resulted in long-term unmaintained remissions (4,5). Our protocol was designed to test the hypothesis that very intensive combination chemotherapy using effective agents in sequential courses, but without long-term maintenance therapy and without marrow rescue, can give prolonged disease-free remissions. We report a 75% CR rate after a single induction course, with an observed disease-free survival rate of 67% (14 ± SE) at 2 years and a projected disease-free survival rate of 38% (17 ± SE) at 4 years.

METHODS

Twelve consecutive previously untreated patients < 50 years of age with AML admitted to the Shands Teaching Hospital or to the Gainesville Veterans Administration Medical Center between December 1978 and August 1981 were entered in this study. Our protocol excluded patients with myelodysplastic syndrome or chemotherapy-induced leukemia. One patient (58 years of age) was inadvertently entered, failed to respond, but, nevertheless, has been included in the analysis. The diagnosis of AML was based on morphologic examination of peripheral blood and bone marrow preparations. By the criteria of the French-American-British classification, Patients 1–11 had M-1 or M-2 marrows at diagnosis, and Patient 12 had an M-4 marrow. Patient characteristics are shown in table 1.

Our protocol prescribed four courses of intensive chemotherapy (table 2). Serial bone marrow aspiration and biopsy were performed to document the response to therapy. A CR was defined as the absence of symptoms and physical findings of leukemia and < 5% myeloblasts and 10% promyelocytes in a cellular marrow sample. The interval between courses was determined by the return of peripheral counts toward normal (granulocyte count ⩾ 2500/mm^3 and platelet count ⩾ 100,000/mm^3) for at least 1 week. The time required to deliver all four courses varied from 5 to 6 months. After completion of the fourth course, neither maintenance chemotherapy nor immunotherapy was administered. Central nervous system prophylaxis was not given.

The duration of survival was measured from the date of initial diagnosis, and the duration of remission was measured from the date of the bone marrow examination documenting a CR.

The Institutional Review Board approved this proto-

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TABLE 1.—Patient characteristics and clinical course

Patient No.	Age (yrs) at presentation	Sex	Blood values at diagnosis: Hematocrit (%)	Blood values at diagnosis: Wbc count (× 10^3 cells/mm^3)	Blood values at diagnosis: Platelet count (× 10^3 cells/mm^3)	Chemotherapy cycles completed	Response*	Duration of CR (mos)†,‡
1	42	F	27	2.8	284	1-4	CR	40
2	29	F	20	100.1	39	1,2§	CR	69+
3	25	M	24	1.5	24	1-4	CR	66+
4	35	F	10	2.7	148	1,2	CR	11
5	28	M	21	395	52	1	NR	NA
6	58	M	27	9.1	200	1	NR	NA
7	31	M	17	4.8	8	1,2	CR	37
8	16	M	26	16.5	113	1-3	CR	5
9	19	M	22	6.6	147	1	NR	NA
10	20	M	40	8.9	130	1,2	CR	¶
11	44	M	25	6.6	125	1,4	CR	46+
12	28	M	27	30.7	513	1,4	CR	44+

*NR = no response.
†As of April 1, 1985.
‡NA = not applicable.
§Daunorubicin deleted during Course 2 due to cardiac toxicity.
¶Underwent bone marrow transplantation 98 days after achieving CR.

col for human experimentation. Each patient gave written informed consent before entry in the study.

RESULTS

Characteristics for the 12 patients are shown in table 1. Nine men and three women, with a median age of 31 years (range, 16–58), were entered in the protocol.

Remission Induction and Consolidation

Nine of 12 patients (75%) achieved CR after one course of chemotherapy. Four of these nine patients received the three additional courses of intensive chemotherapy as planned. Patient 2 had cardiac arrest and anthracycline-induced congestive heart failure; for this patient, Course 2 consisted of cytarabine only. She has received no further chemotherapy after Course 2. Patient 4 was refractory to all transfused platelets and declined any therapy after Course 2. Patient 7 refused further therapy after Course 2. Patient 8 relapsed between Courses 3 and 4. Patient 10 had an HLA-identical sibling and elected bone marrow transplantation after Course 2. His response duration data are, therefore, censored as of the day of transplantation (98 days after achieving CR).

Duration of Remission and Survival

Four of the nine patients achieving CR are continuously free of disease for 44, 46, 66, and 69 months, respectively. In addition, Patient 10, who underwent marrow transplantation on Day 98, is well 53 months after achieving CR. Three patients relapsed at 5, 11, and 40 months, respectively, after achieving CR. Each died in first relapse. One additional patient relapsed at 37 months and is alive in his third CR 61 months after diagnosis.

Toxicity

During remission induction all patients had variable degrees of nausea and vomiting, and all had reversible alopecia. Three patients had severe mucositis; three, diarrhea; two, gastrointestinal bleeding; and one, ileus. Severe interstitial pneumonia of unknown etiology occurred in two patients. One patient had an anthracycline-induced cardiac arrest and congestive heart

TABLE 2.—Chemotherapy regimen

Drug	Course 1	Course 2	Course 3	Course 4
Daunorubicin	40 mg/m^2/day × 3	40 mg/m^2/day × 3	40 mg/m^2/day × 3	40 mg/m^2/day × 3
Cytarabine	200 mg/m^2/day × 10	200 mg/m^2/day × 7		200 mg/m^2/day × 7
6-Thioguanine		100 mg/m^2 twice/day × 5	100 mg/m^2 twice/day × 5	100 mg/m^2 twice/day × 5
5-Azacitidine			150 mg/m^2/day × 7	

failure after a cumulative dose of 120 mg/m^2 of daunorubicin. She recovered and is asymptomatic. Four septic episodes, three bacterial and one fungal, were documented. Two patients died of sepsis, but both had documented primary refractory leukemia. Two patients had bacterial cellulitis. Uterine bleeding requiring hysterectomy occurred in one patient. All patients had granulocytopenia, with a median duration of 24 days (range, 9-27).

Courses 2-4 were associated with fewer severe side effects but were still toxic. One patient had severe mucositis; one, gastrointestinal bleeding; one, atypical mycobacterial pneumonitis; one, reversible cardiomyopathy after 480 mg/m^2 of daunorubicin; one, severe intrauterine bleeding; one, spontaneous intraocular bleed; and one, cerebral vascular accident of unknown etiology. This patient had residual impairment of memory, but motor function was not affected. No patient died of chemotherapy-induced complications during the intensive consolidation therapy.

DISCUSSION

Our pilot study of short-term intensive therapy produced a 75% CR rate in patients with AML. This result is similar to that of a recently published series (1-3). However, the intent of our pilot study was improvement in remission durability. Our approach was structured about the observation that relapse in AML regularly occurs during maintenance therapy, and that the duration of remission may well depend on the cytoreduction achieved initially rather than the chemotherapy given during remission.

Toxicity associated with our intensive regimen was considerable. However, treatment-related mortality was limited to induction, and the only patients who died of toxic complications had documented primary resistance to chemotherapy. The hematologic toxicity was comparable to that of published series, despite the higher doses of cytarabine in our study. Although we did not observe unusually severe gastrointestinal side effects in our group of 12 patients, a subsequent study within the Southeastern Cancer Study Group (SECSG) noted a high incidence (nine of 27 patients) of gastrointestinal bleeding and/or paralytic ileus associated with high mortality. This experience in a larger group of patients suggests that continuous infusion of cytarabine at 200 mg/m^2 for 10 days may be unacceptable.

Six of nine patients who achieved CR had unmaintained, continuous remissions for >36 months. The bone marrow transplant experience in identical twins suggests that a single chemoradiotherapeutic course during remission can achieve durable disease-free survival in 40% of the patients (6). Our data suggest that similar results in young adults are potentially achievable with intensive short-term chemotherapy without marrow transplant. The treatment protocol described here has been adapted to an SECSG phase III study comparing postinduction therapies involving allogeneic bone marrow transplantation.

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Disturbing Central Nervous System Complications Following Combination Chemotherapy and Prophylactic Whole-Brain Irradiation in Patients With Small Cell Lung Cancer[1]

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Despite high complete response rates in small cell lung cancer (SCLC) with combination chemotherapy, only 10%–20% of patients survive 2 years after diagnosis. A common site of recurrence is the brain, with relapse rates of up to 58% in patients surviving 24 months (1). Investigationally, chemotherapy and prophylactic whole-brain irradiation (PBRT) may be combined with the intent of preventing the emergence of brain metastases and improving disease-free survival. Prophylactic cranial irradiation prevents the emergence of brain metastases in patients achieving a complete response with systemic chemotherapy, but with an uncertain impact on survival. We treated 78 patients with SCLC over a 4-year period. Sixty-four presented with limited disease, without apparent brain metastases. A complete remission was achieved in 21 patients (27%) after combination chemotherapy. PBRT was recommended to 17 complete responders; however, two refused therapy. Severe neurologic toxicity occurred in two of the 15 patients (13%) who received PBRT, leading us to withhold radiation from the four remaining complete responders.

CASE REPORTS

Patient 1

Patient 1, a 60-year-old man, was treated for limited SCLC. He received cyclophosphamide (1000 mg/m^2), etoposide (50 mg/m^2), and vincristine (2.0 mg) (CEV) by rapid iv injection on Day 1, and etoposide (100 mg/m^2) orally on Days 2–5 every 3 weeks. He achieved a clinical complete response after five cycles. Consolidation radiotherapy was administered to the primary lesion followed by PBRT administered by Co 60 at 300 rad/day in ten fractions over 2 weeks. CEV was resumed after completion of PBRT. He presented to the clinic 76 days after completion of PBRT with increasing confusion and memory loss. Within 1 week he became less alert and could no longer speak, walk, or eat. Neurologic evaluation was consistent with a subacute confusional state. Head computerized tomography, spinal fluid examination, electroencephalography, and psychological evaluation failed to identify any abnormalities. Chemotherapy was discontinued, and he was transferred to a nursing home where he gradually improved. He remained confined to a wheelchair, but regained the ability to feed himself and speak coherently. Recurrent metastatic disease was identified 12 months after cessation of therapy. No further chemotherapy was administered. The patient died 26 months from the onset of symptoms with persistent neurologic deficits, 35 months from diagnosis. Postmortem examination was not performed.

Patient 2

Patient 2, a 57-year-old man, presented with extensive SCLC (metastases to the left hilum and bone marrow) and received cyclophosphamide (1000 mg/m^2), doxorubicin (50 mg/m^2), and vincristine (2.0 mg) as iv bolus injections every 3 weeks. RA-233, an investigative dipyridamole derivative, was taken orally daily. A complete clinical response was achieved after six cycles of therapy and PBRT was administered by a 4-MeV linear accelerator in ten fractions (300 rads each) over 20 days. Six weeks after completion of PBRT, he was admitted with severe confusion, disorientation, agitation, and urinary incontinence. There was no fever and no evidence of infectious or metabolic abnormalities. Complete neurologic evaluation (head computerized tomography and spinal fluid examination, including cytology) was unrevealing. He rapidly deteriorated and expired, 28 days after the onset of neurologic symptoms. There was no postmortem examination.

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DISCUSSION

Cranial irradiation may be associated with cerebral radionecrosis, but it is unusual at doses administered for prophylactic therapy. Cox et al (2) treated 40 patients with evidence of brain metastases and reported no neurologic sequelae at doses of 3000–4000 rads. Catane et al (3) and Brereton et al (4) each reported little or no neurologic disability after prophylactic cranial irradiation at similar doses.

The combination of radiation therapy and chemotherapy may increase the risk of tissue damage. Ellison et al (5) described confusion and episodic hemiparesis in two of ten patients who had no evidence of disease at 35+ months. Ataxia and a progressive organic brain syndrome occurred in two others. Their patients received cyclophosphamide, methotrexate, lomustine, and vincristine plus chest and whole-brain radiation therapy. They concluded that the combination of radiation therapy plus nitrosourea administration was responsible for the neurologic problems.

Neurotoxicity has been described as resulting from combined chemotherapy and radiation therapy to nervous tissue. Vincristine commonly causes a mild peripheral neuropathy which is dose-related. However, Cassady et al (6) reported severe and prolonged neurotoxicity in four children who received vincristine and radiation therapy to peripheral nerves. Etoposide has also been reported to enhance radiation toxicity during chemotherapy for SCLC (7). It is therefore possible that the neurologic toxicity that occurred in our patients may have been due to an adverse interaction between chemotherapy and radiation therapy. Vincristine and etoposide are the most likely causative agents, since cyclophosphamide and doxorubicin are not associated with neurotoxicity.

Intensive chemotherapy and combined modality therapy of SCLC are major areas of current clinical research. Although most patients tolerate cranial radiation therapy without severe neurologic sequelae, there have been reports of neurologic toxicity after combination chemotherapy and PBRT. The severe nature of the neurologic deterioration reported after combined modality therapy underscores the need to better understand how each of these treatment methods contributes to the problem. To avoid this disastrous complication, we would defer the use of PBRT outside of a research protocol setting in favor of aggressive periodic restaging using cranial computed tomography.

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Randomized Trial of Doxorubicin Versus Cyclophosphamide in Diffuse Malignant Pleural Mesothelioma[1]

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The effect of doxorubicin and cyclophosphamide in the treatment of diffuse, malignant pleural mesothelioma was evaluated in a randomized study. All patients were treated on an outpatient basis and none had previously received antineoplastic treatment. All patients had a measurable lesion other than pleural effusion. The treatment consisted of doxorubicin at a dose of 60 mg/m^2 every 3 weeks, to a total dose of 550 mg/m^2, or cyclophosphamide at a dose of 1500 mg/m^2 every 3 weeks for 1 year. At disease progression the treatment was changed to the alternate drug. The dose was increased or decreased according to hematologic effects. Thirty of 32 patients were evaluable for response. Remissions were not achieved in any patient. During treatment with doxorubicin, none of the patients developed cardiotoxicity, while one patient developed hemorrhagic cystitis during treatment with cyclophosphamide. Sepsis or bleeding was not observed in either of the treatment arms. Thus, the trial showed no antineoplastic activity of either doxorubicin or cyclophosphamide in the treatment of malignant pleural mesothelioma. [Cancer Treat Rep 69:1431–1432, 1985]

Since the incidence of malignant mesothelioma is predicted to increase considerably in the coming decades (1), it is important to establish the value of the different modalities of treatment in this disease.

In a review of the available data on combination chemotherapy for malignant mesothelioma, Aisner and Wiernik (1) concluded that response to chemotherapy could be obtained in 40% of the patients treated with regimens including doxorubicin and in 25% of the patients treated with regimens including cyclophosphamide. However, the data available concerning the various single agents are very modest (2), and consequently, we conducted a randomized trial to evaluate the antineoplastic effect of doxorubicin and cyclophosphamide as single-drug therapy in malignant mesothelioma.

MATERIALS AND METHODS

All patients in this trial were outpatients, with a performance status better than 2 according to World Health Organization (WHO) criteria (3). All patients had histologically proven, nonresectable, diffuse, malignant mesothelioma in progression. Histopathologic classification was performed by an experienced pathologist at the Finsen Institute and all cases were compatible with the diagnosis of malignant mesothelioma (epithelioid, nine patients; sarcomatous, seven; and mixed, 16). No stratification according to histological subtypes was made. Further criteria for inclusion were measurable disease other than pleural effusion and an expected survival of at least 3 months. Minimal hematologic requirements were a platelet count $> 100{,}000/mm^3$ and a wbc count $> 4000/mm^3$. Liver enzymes such as SGOT, serum LDH, and serum creatinine levels had to be normal, unless the abnormal values were due to the neoplastic disease. The patients were randomized to receive either doxorubicin or cyclophosphamide as single-drug therapy and at disease progression the treatment was changed to the alternate drug.

Doxorubicin and cyclophosphamide were given as an iv bolus injection at a dose of 60 and 1500 mg/m^2, respectively, every 3 weeks. If no myelosuppression was seen, the dose was escalated by 20%. Clinical examinations, hematologic status, and plain chest roentgenograms were performed before initiation of therapy and every 3 weeks except the first 3 weeks, where blood cell counts were performed weekly. The occurrence of CNS metastases was not considered to be progression of disease. Other-

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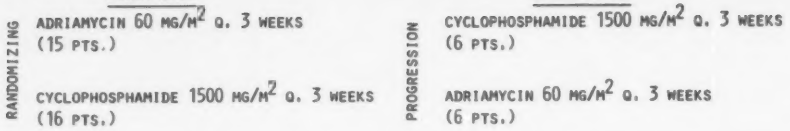

FIGURE 1.—Scheme of treatment.

wise, the evaluation for response was performed according to WHO criteria (3). All patients had given their informed consent with acceptance of the investigational character of the treatment.

RESULTS

Thirty-two patients, with a median age of 56 years (range, 48–68), were included in the trial. None of the patients had previously received chemotherapy or chest irradiation. The median time from diagnosis to start of treatment was 6 months (range, 0–24). Doxorubicin was given to 15 patients as first drug and to six patients as second drug, while cyclophosphamide was given to 16 patients as first drug and to five patients as second drug (fig 1). None of the patients obtained complete or partial remission; all patients had slowly progressive disease. During treatment, none of the patients who received doxorubicin developed signs of cardiotoxicity, while one patient developed hematuria during treatment with cyclophosphamide. Subsequently, the following patients received prehydration with 1000 ml of saline and no further cases of hemorrhagic cystitis were seen.

During treatment with doxorubicin, five patients had dose escalation, while the dose of cyclophosphamide was not escalated in any patient. Sepsis or clinically manifest bleeding was not observed in either of the treatment arms.

DISCUSSION

In this trial we found no antineoplastic activity of either doxorubicin or cyclophosphamide in the treatment of diffuse, malignant pleural mesothelioma. This is in conflict with other trials in which even complete disappearance of the tumor on the chest x-ray has been described (4).

This discrepancy might be explained by the fact that the histological diagnosis can be very difficult to establish.

The growth patterns of the tumor might also explain the discrepancy. The tumor grows as a diffuse sheet of thick, rubbery, yellowish-gray tissue consisting of both collagen and tumor tissue. Because of this, it might be very difficult to assess the regression of the tumor during antineoplastic treatment with available techniques, since it could be concealed by the nonmalignant pleural thickening. The difficulties in assessing the response might be solved by computerized tomographic (CT) scans, since the use of CT density might allow distinction between normal and tumor tissue (5).

In conclusion, we found that single-drug therapy with doxorubicin or cyclophosphamide had no activity in the treatment of diffuse, malignant pleural mesothelioma.

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Treatment of Multiple Myeloma With Recombinant Human Leukocyte A Interferon[1]

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A phase II study of recombinant human leukocyte A interferon was conducted in 64 patients with multiple myeloma in a multi-institutional cooperative trial. Partial remission was achieved in ten (21.3%) of 47 evaluable patients and minor response was observed in five (10.6%). Side effects were noted in more than two-thirds of the patients. They included fever (58%), malaise (20%), anorexia (52%), nausea and vomiting (26%), lethargy (2%), and myelosuppression (56%). An antibody to recombinant human leukocyte A interferon was detected in one of 20 patients. [Cancer Treat Rep 60:1433–1435, 1985]

In addition to antiviral activity, interferon is known to possess antitumor activity against spontaneous and transplantable animal tumors of both viral and nonviral origins (1). Recently, human interferons have been shown to possess antitumor properties against various human malignancies, including multiple myeloma (2–6). Due to their species-specific activities, the early clinical trials have been restricted by the limited supply and high costs of human interferons. The recent progress of recombinant DNA technology, however, has provided large quantities of highly purified single molecular interferons. We report here a result of a phase II study of a recombinant human leukocyte A interferon (rIFN-αA) in a multi-institutional cooperative trial.

PATIENTS AND METHODS

Sixty-four patients with multiple myeloma [42 with IgG, 16 with IgA, five with Bence Jones (B-J) type, and one with IgD] from 21 hospitals were entered in this phase II study. Patients' ages ranged from 39 to 84 years, with a median age of 62 years; there were 39 males and 25 females. rIFN-αA was prepared in *Escherichia coli* by recombinant DNA technology by Hoffmann-La Roche Inc, Nutley, NJ, Takeda Pharmaceutical Company Limited, Osaka, Japan, and Nippon Roche Company Limited, Tokyo. rIFN-αA was administered daily, with doses escalating from 3×10^6 IU to 6, 9, 18, 36, and 50×10^6 IU every 3 days. The maximum tolerable dose was given daily until the side effects made the continuation difficult or until there was no improvement of the disease after at least 4 weeks of treatment. Response was classified as follows (7): *partial response (PR)*—a > 50% reduction of serum M protein and/or a > 50% reduction of urine B-J protein; *minor response*—a 25%–50% reduction of serum M protein and/or urine B-J protein, with the above response sustained at least 4 weeks; *no change*—if M protein remained within ±25%; and *progressive disease*—if M protein increased by > 25%. Antibodies to rIFN-αA were assayed by a plaque reduction assay in FL cells using Sindus virus as a challenge according to the method described by Kohase et al (8).

RESULTS

Among 64 patient entered, 17 were excluded in the evaluation of response because of protocol violations: seven because the preceding antitumor drugs were given within 14 days prior to rIFN-αA; four because of concurrent use of prednisolone; three because their performance scores were 4 (Eastern Cooperative Oncology Group); and three because the administration period of rIFN-αA was < 14 days due to severe side effects. The last three patients were included in the evaluation of side effects. Maximum daily doses given were 50×10^6 IU in five patients, 36×10^6 IU in six, 18×10^6 IU in ten, 12 ×

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TABLE 1.—rIFN-αA treatment of multiple myeloma

Type of myeloma	No. of patients	Response*				Response rate (%)	
		PR	MR	NC	PD	PR	PR + MR
IgG	31	5	2	23	1	16.4	22.5
IgA	12	4	3	5	0	33.3	58.3†
B-J	4	1	0	3	0	25.0	25.0
Total	47	10	5	31	1	21.3	31.9

*MR = minor response; NC = no change; and PD = progressive disease.
†$0.05 < P < 0.1$.

10^6 IU in three, 9×10^6 IU in 11, 6×10^6 IU in eight, and 3×10^6 IU in four. The treatment with the doses of $\geq 12 \times 10^6$ IU was not well-tolerated for a long time because of side effects such as malaise, anorexia, and fever, and the median treatment periods of the patients given 50, 36, 18, and 12×10^6 IU were 13, 5, 8, and 13 days, with ranges from 5 to 25, 2 to 13, 3 to 33, and 7 to 18 days, respectively. The usual maintenance daily doses given in the responding patients were 9 or 6×10^6 IU. The treatment period ranged from 14 to 345 days, with a median of 36 days. Total doses given ranged from 72 to 3526 $\times$ 10^6 IU, with a median of 252×10^6 IU.

Among 47 evaluable patients, ten achieved PR, five had minor response, 31 had no change, and one had progressive disease. The response rate was 21.3%, and if minor response was included, 31.9% (table 1). Seven patients, who were excluded in the evaluation of response because of prior chemotherapy within 14 days, had no change if evaluated. The response according to type showed no particular pattern of sensitivity. However, when minor response is included, IgA tended to show a better response (58.3%) than IgG (25.8%) and B-J (25%) ($0.05 < P < 0.1$ by chi-square test with Yates' correction). To achieve PR, a median of 29 days (range, 22–89) was required and a median total dose of 532×10^6 IU (range, 84–916 $\times$ 10^6) was given. The PR lasted for a median of 8 weeks (range, 4–55). In two patients PR lasted for 55 and 45+ weeks. In table 2, the median total dosage, the median treatment period, and the median and mean daily dose are shown. Patients achieving PR received higher doses of rIFN-αA per day ($P < 0.01$ by t test). Thirty-nine patients had previously been treated with various chemotherapeutic drugs and had been considered clinically refractory to these drugs, and eight were previously untreated. PR was achieved in 20.5% of previously treated patients and in 25% of previously untreated patients with less advanced disease. Side effects were noted in more than two-thirds of the 50 patients evaluated (table 3). Fever seemed to be unrelated to the doses of rIFN-αA and became uncommon after several days' treatment. Anorexia, nausea and vomiting, and malaise seemed to be related to the doses of rIFN-αA and became dose-limiting factors in some patients who received high doses. Myelosuppression was common, but returned quickly to the pretreatment values when rIFN-αA was discontinued. Mild elevation of transaminases was observed in 20%. Elevation of BUN was noted in one patient whose kidney function had already been impaired. Antibody to rIFN-αA was tested in 20 patients at 2–4-week intervals. A neutralizing antibody was detected in one patient 11 weeks after the initiation of rIFN-αA. No particular reaction to rIFN-αA was observed clinically in this patient.

DISCUSSION

rIFN-αA was found to be effective for multiple myeloma. PR was achieved in ten of 47 evaluable patients (21.3%). If patients with minor response are included, the response was observed in 17 patients (31.9%). The response rate of multiple myeloma to rIFN-αA seems to be almost the same as that to leukocyte α-interferon and human fibroblastoid β-interferon (4,5). Although we tried to give maximum tolerable doses of rIFN-αA, the longer use of the higher doses of rIFN-αA such as 36 or 50×10^6 IU/day was almost intolerable to patients due to severe malaise and anorexia. For most of the patients, 9×10^6

TABLE 2.—Dose of rIFN-αA related to response

Response*	No. of patients	Median total dose ($\times 10^6$ IU)	Median treatment time (days)	Daily dose ($\times 10^6$ IU)	
				Median	Mean
PR	10	891	38	12.0	16.0†
MR	5	141	38	5.4	7.0
NR (NC + PD)	32	228	35	7.6	8.1

*MR = minor response; NR = no response; NC = no change; and PD = progressive disease.
†$P < 0.01$ vs NR and not significant vs MR.

TABLE 3.—Side effects of rIFN-αA treatment

	No. of patients (%)
Fever > 38°C	29 (58)
Anorexia	26 (52)
Nausea and vomiting	13 (26)
Malaise	10 (20)
Epilation	2 (4)
Dyspnea with cyanosis	1 (2)
Lethargy	1 (2)
Paresthesia	1 (2)
Thombocytopenia *	
> 25%	28 (56)
> 50%	19 (38)
Leukopenia *	
> 25%	23 (46)
> 50%	11 (22)
Elevation of SGOT/SGPT	10 (20)
Elevation of BUN	1 (2)
Proteinuria	2 (45)

* > 25% or 50% reduction of the pretreatment values.

IU of rIFN-αA was the maximum tolerable daily dose for longer use. However, higher doses seemed to exert more effect, since ten patients achieving PR received a mean daily dose of 16.0×10^6 IU, while five with minor response and 32 nonresponders received 7.0 and 8.1×10^6 IU, respectively ($P < 0.01$). This indicated that patients who could tolerate higher doses of rIFN-αA or those who were administered higher doses despite side effects had a better chance of responding. The side effects of rIFN-αA were almost the same as those reported in previous studies on interferons (2-6). Neurological toxicity was observed in two patients. rIFN-αA was effective for the patients who had been treated with melphalan and other antitumor drugs and had become clinically refractory to these drugs. Therefore, at least clinically, there seemed to be no cross-resistance between these drugs. The results of the present study indicate a definite efficacy of rIFN-αA for multiple myeloma and warrant further study of this agent, especially in combination with other antitumor drugs.

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Salivary Hyperamylasemia After L-Asparaginase Therapy[1,2]

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L-Asparaginase is an effective drug in the treatment of leukemia (1,2). However, its use has been associated with many side effects, including acute pancreatitis (3–5). We describe a patient who developed hyperamylasemia during L-asparaginase therapy, but who did not develop signs or symptoms suggestive of pancreatitis. The elevated serum amylase activity in this patient subsequently was shown to be predominantly salivary isoamylase. This case demonstrates the importance of determining the origin of hyperamylasemia in patients treated with L-asparaginase, as it may influence the continued use of this drug.

CASE REPORT

The patient was a 40-year-old male with acute lymphoblastic leukemia, admitted to the Albert B. Chandler Medical Center of the University of Kentucky for bone marrow transplantation (UKBMT UPN No. 9). He had been in good health until 3 months prior to admission, when he presented to another hospital with complaints of headache, fatigue, and dyspnea on exertion. Laboratory investigation was remarkable only for anemia and leukopenia. Bone marrow aspiration and biopsy were performed with findings consistent with acute lymphoblastic leukemia. Induction chemotherapy was begun, consisting of vincristine, doxorubicin, and prednisone. This was followed by five doses of L-asparaginase (40,000 U each) and intrathecal methotrexate administration. The patient tolerated induction chemotherapy well; consolidation chemotherapy was begun, which consisted of doxorubicin, vincristine, and prednisone. A repeat bone marrow biopsy, after completing two courses of consolidation chemotherapy, was consistent with remission. Upon admission to the University of Kentucky Medical Center, the patient was asymptomatic, on no medications, with his leukemia in remission. Review of systems revealed no personal or family history of pancreatitis or biliary disease, and no alcohol or drug abuse. There had been no previous surgery. Physical examination was unremarkable. Laboratory tests revealed a hemoglobin of 13.1 g/dl, a wbc count of 9700/mm^3, a platelet count of 279,000/mm^3, fibrinogen of 325 mg, prothrombin time of 11.5 secs (control, 12.5), and partial thromboplastin time of 27.1 secs (control, 22). Serum electrolytes, as well as total bilirubin, alkaline phosphatase, SGOT, and SGPT, were normal. Serum amylase was 31 U/L (normal, 30–120). Urinalysis was unremarkable and chest x-ray was normal. A repeat bone marrow biopsy on admission was again consistent with remission. On Day 2 of hospitalization, the patient began a 10-day course of combined chemotherapy and radiotherapy in preparation for his bone marrow transplant, which consisted of L-asparaginase daily for 6 days (15,000 U/m^2 of body surface area), methylprednisolone, cyclophosphamide, and total-body irradiation.

On Day 6 of L-asparaginase therapy, the patient's serum amylase had risen to 1550 IU/L. Figure 1 depicts the patient's serum amylase values in relation to L-asparaginase therapy. The full course of L-asparaginase was given without interruption. During the course of hyperamylasemia, the patient had no abdominal pain, nausea, or vomiting. He tolerated oral feedings well. Abdominal examination remained unremarkable, and there was no evidence of parotid swelling. After L-asparaginase was discontinued, the hyperamylasemia resolved. The serum lipase concentration, drawn while the serum amylase was elevated, was 0.24 U/dl, with a normal value of $\leq$ 1.0 U/dl. Isoamylases were separated by column chromatography (6). The elevated serum amylase activity was found to be predominantly salivary isoamylase. The patient was eventually discharged in good health on maintenance prednisone therapy for graft versus host disease prophylaxis.

DISCUSSION

The antineoplastic effects of L-asparaginase have been related to specific depletion of L-asparaginase, resulting in death of neoplastic cells which lack the endogenous ca-

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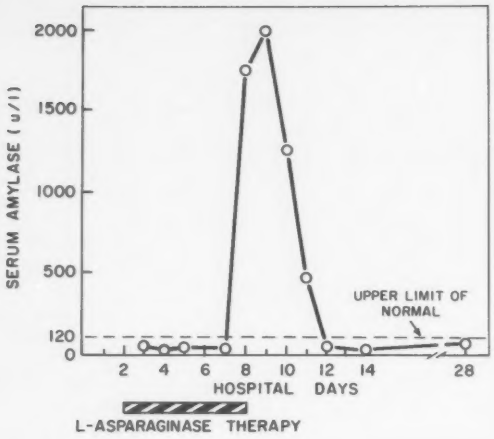

FIGURE 1.—Graph depicts the patient's hyperamylasemia in relation to his hospitalization and L-asparaginase therapy.

pacity to synthesize L-asparagine (7). L-Asparaginase toxicity in man may be related to four different factors: (*a*) contamination of the preparation with *Escherichia coli* endotoxin; (*b*) contamination of the preparation with L-glutaminase activity; (*c*) hypersensitivity; or (*d*) generalized inhibition of protein synthesis. Organs with high rates of protein synthesis which utilize extracellular L-asparagine in their metabolism, such as the pancreas and the liver, appear to be predisposed to L-asparaginase toxicity.

Acute pancreatitis has been reported as a complication of L-asparaginase therapy, with a mean incidence of 7% (range, 0%–25%) (1–5,8,9). The onset of pancreatitis has varied from 3 days after beginning therapy to 10 weeks after termination of therapy, with a median of 6 days after beginning therapy (5). The severity of this pancreatitis ranges from mild to severe, with several reports of hemorrhagic pancreatitis (4,5,10). The reported mortality rate due to this form of pancreatitis has varied from 1.8% to 4.6% in three studies totaling 203 patients. Autopsy studies have demonstrated occult pancreatitis in 1.9%–7.3% of patients who had received L-asparaginase (3–5). Long-term complications, such as pseudocyst formation, have also been reported (10).

The high incidence of unequivocal acute pancreatitis associated with L-asparaginase therapy strongly suggests a causal relationship. However, no L-asparaginase challenge has been reported in a patient felt to have developed pancreatitis from this drug. Also, the baseline incidence of acute pancreatitis in a matched control population is not known. Thus, the association is probable but remains unproven.

To our knowledge, this is the first report of salivary hyperamylasemia associated with L-asparaginase therapy. There have been previous reports of "pancreatitis" and hyperamylasemia developing during L-asparaginase administration in which postmortem examination revealed a morphologically and histologically normal pancreas (5). However, lipase and isoamylase determinations were not performed in these patients. The pancreas and parotid glands have many structural and functional similarities, and an agent with known pancreatic toxicity, such as L-asparaginase, also might produce parotid injury. This observation may prove to be of therapeutic importance, as L-asparaginase is regularly discontinued when a diagnosis of acute pancreatitis is made during L-asparaginase administration. However, our patient was asymptomatic during the period of hyeramylasemia, and he tolerated his full course of L-asparaginase therapy without difficulty. It remains to be determined whether asymptomatic salivary hyeramylasemia should influence continued L-asparaginase therapy.

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Effectiveness of Acute Nonlymphocytic Leukemia Induction Chemotherapy in Acute Lymphocytic Leukemia[1,2]

Peter A. Cassileth[3,*] and John M. Bennett[4]

Twelve adults with newly diagnosed acute lymphocytic leukemia (ALL) received a combination of daunorubicin, cytarabine, and 6-thioguanine (DAT) as induction chemotherapy. Eleven patients (91%) gained complete remission (ten patients after a single course of treatment). Because DAT alone seems effective in ALL, the combination of vincristine, prednisone, and DAT could improve the complete remission rate. Moreover, the resulting early leukemic cytoreduction could enhance remission duration in adults with ALL. [Cancer Treat Rep 69:1439–1440, 1985]

Standard induction chemotherapy for adults with acute lymphocytic leukemia (ALL) consists of vincristine, prednisone, and an anthracycline with or without L-asparaginase (1), with complete remission rates ranging from 70% to 85% (2–4). Most induction chemotherapy programs for adult acute nonlymphocytic leukemia (ANLL) consist of daunorubicin and cytarabine with or without 6-thioguanine (DAT) (5). We briefly present here the results of DAT induction chemotherapy in adult patients with ALL.

METHODS

The Eastern Cooperative Oncology Group (ECOG) utilizes a central reference laboratory (directed by J. M. Bennett) to confirm the morphologic diagnosis of all patients entered in acute leukemia protocols. Bone marrow aspirates and/or peripheral blood films are examined after Wright-Giemsa and cytochemical stains to classify acute leukemia according to the French-American-British classification scheme (6). Two recent ECOG protocols (EST 1479 and PB482) used the identical (DAT) induction chemotherapy regimen consisting of daunorubicin (60 mg/m^2/day on Days 1, 2, and 3), plus cytarabine (25 mg/m^2 iv push followed by continuous infusion of 200 mg/m^2/day on Days 1–5), and 6-thioguanine (100 mg/m^2 every 12 hours orally on Days 1–5). Of the 402 patients entered in these two studies, 12 (nine of 312 on EST 1479 and three of 90 on PB482) were reclassified as ALL after review. All of these patients had received DAT induction chemotherapy according to protocol before the individual investigators received notice of the change in diagnosis. Follow-up information on these 12 patients was obtained for the purpose of this report.

RESULTS

The 12 patients ranged in age from 19 to 62 years (median, 40). Morphologic classification showed that five patients were L1 and seven were L2. Eleven of 12 patients (91%, with a 95% confidence interval of 76%–100%) gained complete remission, ten after a single course and one after two courses of the induction regimen. The time to complete remission ranged from 22 to 45 days (median, 27). There were no deaths from DAT induction therapy. One of the two patients, who did not obtain complete remission after two courses of induction therapy, then received vincristine and prednisone induction and gained a complete remission which continues for > 26 months.

Subsequent to obtaining complete remission, most of these patients were treated with maintenance regimens considered to be appropriate for ALL, and most received prophylactic cranial irradiation and intrathecal methotrexate. Two of the 11 patients who obtained complete remission after DAT remain in continuous complete remission at 12+ and 46+ months. The median duration of remission is > 12 months (range, 1–50). Two other patients are alive in second complete remission, both at 14+ months from diagnosis.

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DISCUSSION

The results of therapy of these 12 patients with ALL with the DAT regimen used in ANLL suggest that DAT is a highly effective induction therapy regimen in ALL as well. The patients with ALL reported here were inadvertently entered on an ANLL protocol by ECOG investigators. Therefore, it is difficult to be certain that these patients are representative of all adult patients with ALL. Nevertheless, ten of the 11 patients required only a single course of induction chemotherapy, and the median time to complete remission was 4 weeks. In adults with ALL, Gottlieb et al (2) have shown that daunorubicin is needed in addition to vincristine and prednisone to achieve a high complete remission rate. However, even with daunorubicin, vincristine, and prednisone, and L-asparaginase induction therapy, 25% of adults with ALL require > 4 weeks and additional drug therapy to obtain complete remission (4). Earlier reports noted some activity for the combination of cytarabine and 6-thioguanine in ALL (7). A combination of DAT with vincristine and prednisone could therefore enhance remission induction. Moreover, it has been shown that early rapid cytoreduction in ALL is associated with an increased likelihood of remission induction (8) and an increased duration of complete remission (4,8). A low total dose of doxorubicin plus cytarabine, vincristine, and prednisone has been shown to yield a high remission rate (9). The combination of DAT plus vincristine and prednisone, using higher doses of daunorubicin than ordinarily employed in ALL (60 vs 45 mg/m^2) plus the addition of cytarabine and 6-thioguanine, could well facilitate more rapid early destruction of the leukemic cell population. The increased myelosuppression produced by this regimen would be justified if the outcome is enhanced remission duration.

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Ultrafiltrate and Total Platinum in Plasma and Cerebrospinal Fluid in a Patient With Neuroblastoma[1,2]

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Ultrafiltrate and total plasma and cerebrospinal fluid (CSF) platinum concentrations were examined in a patient with neuroblastoma following a 2-hour infusion of 120 mg/m² of cisplatin. Total plasma platinum was 93.4% bound, while the ultrafiltrate of plasma accounted for the remaining 6.6%. The CSF ultrafiltrate resulted in equivalent concentrations of platinum to total CSF platinum. CSF concentrations were 2.9% of the total plasma platinum and 43.5% of the ultrafiltrate plasma platinum 2 hours after the end of the infusion. [Cancer Treat Rep 69:1441–1442, 1985]

Cisplatin is one of the most important chemotherapeutic agents currently available for the treatment of patients with a variety of cancers (1). In addition to the toxic effects of cisplatin therapy (auditory, stomatitis, alopecia, renal), reports of peripheral neuropathies and drug-induced seizures suggest that platinum penetrates into the cerebrospinal fluid (CSF) (2,3). Khan et al (4) have shown additional evidence that cisplatin penetrates into the CSF by demonstrating that patients with brain tumors have responded to iv cisplatin therapy.

Presently, we examined the total and ultrafiltrate platinum in plasma and CSF specimens after a 2-hour infusion of cisplatin in a patient with neuroblastoma.

Patient History

The patient is a 2-year-old white male with frontotemporal cerebral neuroblastoma who had prior surgical resection of the tumor followed by full craniospinal radiation therapy. Prior to this admission, an Ommaya reservoir was implanted sc for previous chemotherapy administration which included vincristine and mechlorethamine. At this time, the patient had normal renal function and had 46 mg/dl of protein in the CSF (normal limits, 15–45 mg/dl). Single-agent systemic cisplatin therapy was administered at a dose of 120 mg/m² by a 2-hour infusion. Blood samples were collected from an indwelling catheter while CSF samples were collected from the Ommaya reservoir.

MATERIALS AND METHODS

Blood and CSF samples were collected within 5 minutes of each other at 0.25, 2, 4, 24, and 48 hours after a 2-hour infusion of 120 mg/m² of cisplatin. Blood samples were anticoagulated with EDTA and rapidly cooled to 4°C. Each blood sample was then centrifuged for 10 minutes at 2000 rpm to remove rbc and lymphocyte contamination. Ultrafiltrate platinum was separated from plasma and CSF using Amicon CF30 filters with centrifugation at 5000 rpm for 20 minutes. All samples were then stored at -20°C until analysis.

Quantitation of platinum levels was performed utilizing a flameless atomic absorption spectrophotometer. To summarize, a Perkin Elmer model 2280 atomic absorption spectrometer set for 266.9-nm absorption equipped with a Perkin Elmer HGA 400 graphite furnace was standardized with stock cisplatin solutions (donated by Bristol Laboratories, Syracuse, NY), which resulted in reproducible linearity through the desired concentrations ($r = 0.994$). Triplicate samples (20 μl) of total plasma, ultrafiltrates of plasma, total CSF, and ultrafiltrates of CSF were injected for analysis.

RESULTS

The CSF and plasma concentrations of platinum are shown in figure 1. No differences were seen in the platinum concentrations of CSF ultrafiltrates when compared

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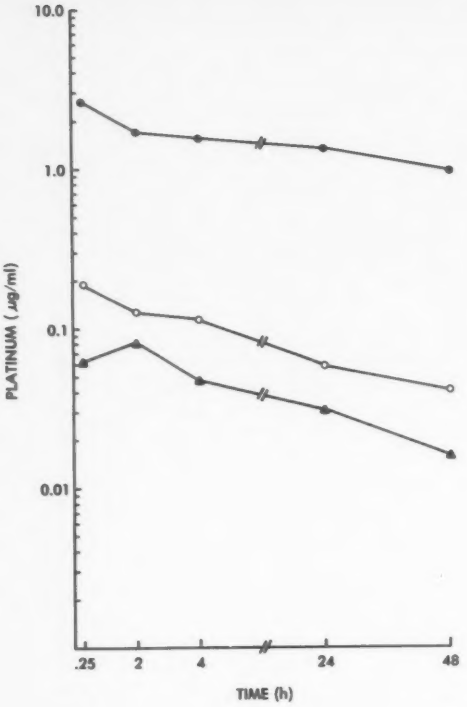

FIGURE 1.—Total plasma (●——●), ultrafiltrate of plasma (○——○), and CSF (▲——▲) platinum levels after a 120-mg/m^2 dose of cisplatin. Each point is the mean of triplicate samples.

to those of total CSF platinum during the study period. Total plasma platinum resulted in 93.4% protein bound, while 6.6% was present in plasma ultrafiltrate. CSF levels of platinum peaked between 15 minutes and 2 hours after the cessation of the cisplatin infusion. The terminal half-life of elimination for total plasma, ultrafiltrate of plasma, and CSF platinum was 56.8, 34.4, and 33.3 hours, respectively. CSF levels of platinum were approximately 43.5% of the unbound plasma levels which represented 2.9% of total plasma platinum (vol:vol) 2 hours after the end of the cisplatin infusion.

DISCUSSION

Experimental data in rhesus monkeys show that the ratio between CSF platinum and total plasma platinum is approximately 0.04 (4%) after an iv administered dose of cisplatin (5). Similar to our results, Higby et al (6) reported a single patient who had 2.5% of total plasma platinum penetrate into the CSF (vol:vol) after a single dose of cisplatin.

Interestingly, we showed a long terminal half-life of elimination for plasma ultrafiltrate and CSF elemental platinum. It is important to note that between 2 and 4 hours after the end of a cisplatin infusion, other inactive platinum complexes probably exist and may account for the majority of ultrafiltrate plasma platinum species (7). In addition, the ultrafiltrate plasma platinum detected < 2 hours after the end of the infusion probably represents the active cisplatin complex (7).

Several investigators suggest that the unbound fraction of cisplatin reflects the active portion of the total plasma platinum (8). Since the CSF of our patient had a relatively minor amount of protein, we were not surprised to find equivalent concentrations of platinum in both total and ultrafiltrates of CSF. Therefore, total CSF levels of platinum probably reflect the active portion of the drug within 2 hours after the cessation of the cisplatin infusion.

Our results show that CSF levels of platinum are approximately 25%–43.5% of the ultrafiltrate plasma concentration as compared to 2%–3% of total platinum. In addition, the terminal half-life of elimination of CSF platinum was similar to that of ultrafiltrate plasma platinum. Since the effects of whole-brain irradiation and cranial surgery on the penetration of cisplatin into the CSF remains unclear, we cannot determine whether the CSF platinum levels seen in our patient were influenced by these procedures. However, if CSF levels of platinum can be estimated from the plasma platinum kinetics, then cytotoxic levels of cisplatin can potentially be targeted to the CSF. Further research examining the relationship between ultrafiltrate CSF platinum and plasma platinum kinetics is warranted.

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Circadian Rhythm in Tolerance of Mice for Etoposide[1,2]

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Etoposide (40 mg/kg/day × 3 days and 60 mg/kg/day × 3 days) was best tolerated by male B6D2F$_1$ mice when given in the second half of the rest span of their sleep-wake circadian cycle. Such a time-qualified treatment resulted in increased long-term survival rate, highest peripheral leukocyte count at nadir, and lowest body weight loss, as compared to results from drug dosing in the activity span. Assuming that such results may be extrapolated to human beings, the treatment time of etoposide associated with an optimal tolerance would be located in the second half of the sleeping span (usually near 5.00 hrs). [Cancer Treat Rep 69:1443–1445, 1985]

Murine tolerance for more than ten anticancer drugs depends upon their dosing time in the 24-hr scale (1). The relevance of the mouse model to predict for the optimal dosing time of doxorubicin in cancer patients has already been demonstrated (2).

The increasing clinical use of the epipodophyllotoxin derivative etoposide (VP16) prompted the present studies. The mouse model was used to investigate whether drug-induced lethality and hematologic toxicity were affected by the circadian time of injection of this drug.

MATERIALS AND METHODS

Two studies were performed in male B6D2F$_1$ mice kept in an artificial lighting regimen consisting of an alternation of 12 hrs of light and 12 hrs of darkness for 3 weeks prior to and throughout each study.

VP16 (dry powder) was mixed in a 1:9 solution of Tween 80 (TW80) and a 0.9% NaCl solution. All mice received an iv injection into the retro-orbital sinus daily for 3 consecutive days.

In Study I (4/25/84), 85 19-week-old mice received a total dose of 120 mg/kg of VP16 (40 mg/kg/day × 3 days). Thirty-six control mice received the vehicle alone (TW80:saline, 1:9). Six dosing times were explored, with reference to light onset (onset of the resting span): 3, 7, 10, 14, 19, and 23 hours after light onset (HALO). Survival rate was recorded daily and body weight loss measured two to three times weekly for 60 days. Leukocyte count was determined 5 days after first dosing in separate subgroups of mice near both the peak (6 ± 4 HALO) and the through (18 ± 4 HALO) of its physiologic circadian rhythm in mice (3).

In Study II (5/16/84), 33 13-week-old mice received a total dose of 180 mg/kg of VP16 (60 mg/kg/day × 3 days). Two dosing times were compared: 7 HALO and 19 HALO. The observation lasted 40 days. The endpoints included daily survival rate and body weight loss measured two to three times weekly.

The chi-square test (Yates-corrected, when required) and/or t test and/or single cosinor analysis (4) were performed. This linear least-squares method provides a *P* value from an F test of the rejection of the null-amplitude hypothesis, and rhythm characteristics with their 95% confidence limits. These include the mesor (the 24-hr-rhythm-adjusted mean of the best-fitting cosine function), the double amplitude (difference between maximum and minimum of best-fitting cosine function), and the acrophase (time of maximum of best-fitting cosine function) referred to light onset.

RESULTS

Overall Toxicity

Early mortality was observed in both studies. Thus, six

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[5]We thank Guy Lemaigre for the histologic studies of the lungs and Elisabeth Couvé and Nicole Vriz for excellent editorial assistance.

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of 85 mice (7%) in Study I and eight of 33 mice (24.2%) in Study II died before completing the 3-day injection course. Autopsy (of these early deaths) revealed massive lung infarctions, and lung histology indicated a major acute pulmonary alveolitis. Subsequent deaths were recorded between Days 5 and 32. The overall survival rate at study completion was 80% in Study I and 36.3% in Study II. Body weight loss (as percent of initial body weight) was maximal on Day 5 in both studies (mean ± 1 SEM): 16.0 ± 0.6% in Study I and 19.4 ± 1.1% in Study II. In Study I, mean leukocyte count (cells/mm^3 ± 1 SEM) on Day 5 was 6040 ± 400 in controls and 1690 ± 90 in VP16-treated mice, suggesting that bone marrow suppression largely contributed to the second wave of mortality.

Time-Dependent Toxicity

In Study I, the survival rate and maximal body weight loss varied as a function of dosing time. A statistically significant circadian rhythm in the leukocyte count of control mice was demonstrated with cosinor ($P < 0.001$; mesor = 6040 ± 400; double amplitude = 5820 ± 1920; acrophase = 02:00 ± 02:50 HALO), similar to that reported by others (3). Therefore, the mean leukocyte counts of mice treated at different circadian stages were expressed as percentages of their respective controls. A statistically significant circadian rhythm was validated by cosinor for both body weight loss and leukocyte count, with similar acrophases (fig 1). Such results indicated that optimal tolerance for VP16 injected iv was achieved by administering this drug between 7 and 11 HALO.

In Study II, nine of 17 mice (53%) were alive 40 days after etoposide at 7 HALO, as compared to three of 16 mice (18%) after dosing at 19 HALO ($\chi^2 = 4.0$; $P < 0.05$). Maximal body weight loss (mean ± 1 SEM) was larger in the group treated at 19 HALO (21.3 ± 2.1%) than in the group treated at 7 HALO (17.7 ± 0.7%) (t = 1.7; P = 0.10).

DISCUSSION

VP16, in the preparation used, exhibited two major toxic effects which appeared to be mainly targeted at the lung (initial mortality) and at the bone marrow. The pulmonary alveolitis which was documented most likely reflected a "colloidal shock," secondary to the difficulty in obtaining a preparation of VP16 with low diameter particles. This has already been reported (5).

Nonetheless, a circadian rhythm was demonstrated for murine tolerance for etopside, given iv daily for 3 consecutive days. Both hematologic tolerance and survival rate were increased and body weight loss was minimized by administering VP16 in the second half of the light (rest) span of mice.

Such a timing was also found to result in highest survival rates in CD2F$_1$ mice treated with VP16 given as a single sc injection (6). Thus, neither the route nor the schedule of injection seems to alter the circadian pattern of tolerance for this drug.

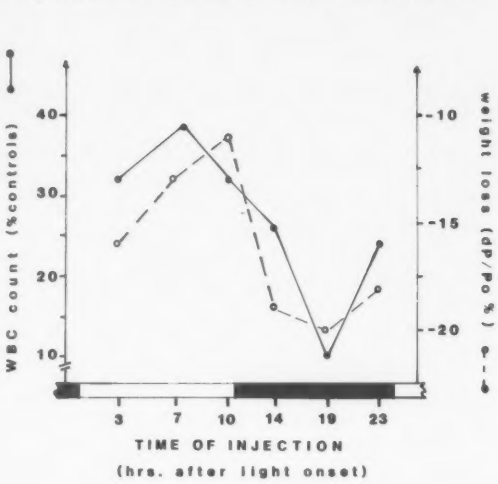

FIGURE 1.—Mean body weight loss and mean leukocyte count 5 days after first dosing of VP16 (40 mg/kg/day × 3 days, iv). Body weight loss (dP) was expressed as a percentage of body weight measured on the day of first injection (PO). Mean leukocyte count of treated mice (14/time point) was expressed as a % of time-qualified mean control value (6 mice/time point). Cosinor analysis validated a circadian rhythm for both variables ($P < 0.05$). The double-amplitude and acrophase (± 1 SD) were, respectively, 41% ± 12% of the mesor and 9.20 ± 2.50 hrs for body weight loss, and 99% ± 40% of the mesor and 7.20 ± 4.20 hrs.

Since the optimal dosing time for other myelosuppressive agents is also located in the second half of the light span (1,7), the circadian rhythm in bone marrow tolerance for such cytostatics may depend upon that in bone marrow proliferation, rather than that in drug pharmacokinetics. Taking into account the properties of biologic rhythms, our results may predict for an optimal hematologic tolerance of VP16 near 5.00 hrs (second half of the sleeping span) in man.

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Amsacrine With High-Dose Cytarabine in Acute Leukemia[1]

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Amsacrine (AMSA) has been proposed at doses > 450 mg/m^2/course for the treatment of patients with acute leukemia resistant to anthracyclines (1). High-dose cytarabine (HD-ARAC) (3 g/m^2 every 12 hours for 2-6 days) has been given to overcome resistance to cytarabine (2), but the usual 6-day courses induced cutaneous and cerebellar toxicity. Therefore, we administered a combination of AMSA and 2 days of HD-ARAC to patients with relapsing and/or refractory acute leukemia.

PATIENTS AND METHODS

Sixty-five patients with relapsing or refractory acute leukemia, mainly with acute nonlymphoblastic leukemia (ANLL), were treated. The drugs were infused iv over 1 hour in separate catheters: AMSA was given at a dose of 90-120 mg/m^2/day for 5 days, and HD-ARAC was given at 3 g/m^2 twice a day for the first 2 days (four doses). Four patients received two courses of treatment, and one received three, at 2-4-week intervals.

Results were classified as complete remission (CR), partial remission (PR), death during treatment or consecutive hypoplasia, and resistant disease with persisting leukemia.

RESULTS AND DISCUSSION

The response rate was 43%, with a 36% CR rate in the 47 patients with ANLL. A 26% CR rate was also obtained in patients refractory to anthracyclines plus conventional doses of cytarabine (tables 1 and 2). These CR were obtained after one course (except in two patients who received two courses); median time to CR was 29 days (range, 18-90). Median duration of CR was 7 months (range 2-38+). Two patients are surviving after allogeneic bone marrow transplantation, and one is surviving after 38 months of maintenance treatment with AMSA plus HD-ARAC.

Toxicity was mainly hematologic; 17% of the deaths occurred during hypoplasia (oral, digestive, and hepatic). Median duration of neutropenia was 19 days in patients who were in remission. No ocular, cutaneous, or cerebellar toxicity was observed. Cardiac toxicity (preexistent in one patient) was observed in two patients who had been pretreated with anthracyclines.

Our results in refractory ANLL could be superior to those obtained with either AMSA alone (1), or 6-day courses of HD-ARAC (2). Recently, Hines et al (3) reported a 70% CR rate in relapsing ANLL with 6-day courses

TABLE 1.—Patient responses

Cytological type*	Median age in yrs (range)	No. of patients				
		CR	PR	RD†	Death	Total
ANLL	49 (10-72)	17	3	18	9	47
	≤ 20	4	0	1	0	5
	21-40	4	3	5	2	14
	41-60	6	0	10	4	20
	> 60	3	0	2	3	8
ALL	28 (10-56)	3	0	9	2	14
BC-CML	50 (39-58)	0	0	4	0	4

* ALL = acute lymphoblastic leukemia; BC-CML = blast crisis-chronic myelocytic leukemia.
†RD = resistant disease.

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[2]The European Organization for Research on Treatment of Cancer Leukemia-Lymphoma Group.

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TABLE 2.—Treatment history of patients with ANLL

Treatment history	No. of patients				
	CR	PR	RD*	Death	Total
1st induction failure	6	1	7	5	19
1st relapse	8	1	4	3	16
≥ 2nd relapse	3	1	7	1	12
Total refractory	13	1	14	8	36

*RD = resistant disease.

of HD-ARAC plus AMSA (100 mg/m^2 on Days 7-9), but their response rate in refractory ANLL was not mentioned.

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Use of Protein A-Sepharose-Treated Sera in Human Acute Nonlymphocytic Leukemia: Lack of Toxicity[1,2]

Thomas R. Spitzer,* Hillard M. Lazarus, and Lee A. Biblo[3]

Plasma or serum treated with staphylococcal protein A has been reported to have tumoricidal properties when infused into tumor-bearing humans or animals (1,2). We recently reported that an inhibitory factor may be present in leukemic sera which impairs the mitogenesis of remission lymphocytes when exposed to autologous leukemic blasts, and that this factor can be effectively removed by protein A (3). Based on these data, we re-infused serum treated with protein A-Sepharose into two patients with acute nonlymphocytic leukemia resistant to intensive chemotherapy. The lack of toxicity observed with seven treatment courses is consistent with the previously demonstrated absence of protein A in protein A-Sepharose-treated sera (3).

MATERIALS AND METHODS

Two patients with refractory acute nonlymphocytic leukemia underwent multiple phlebotomies of 500–1000 ml of whole blood. Fifty-milliliter aliquots of autologous sera or plasma were mixed with protein A-Sepharose (Pharmacia Fine Chemicals, Sweden) at a concentration of 30 mg of protein A/ml of serum and filtered through 0.22-micron Millex-GV filters (Millipore, Bedford, MA). Various volumes of serum or plasma were then infused through a peripheral iv line (table 1). Research nurses obtained blood pressure, pulse, temperature, and respiratory rate before the start of the infusion and every 15 minutes during the infusion, and recorded results.

RESULTS

Treatment with protein A-Sepharose reduced IgG concentrations by 522 and 497 mg/dl in the two patients' sera. We observed no toxicity (fever, chills, change in systolic or diastolic blood pressure of > 15 mm of mercury or change in pulse rate of > 10/min) with re-infusion of protein A-treated serum. Infusion rates were comparable to those reported in other human studies in which marked toxicity was observed. Likewise, antileukemic effects as evidenced by reduction in percent of leukemic blasts in the bone marrow or improvement in peripheral counts were not observed. In Patient 1, the percent of marrow leukemic blasts positive for surface immuno-

TABLE 1.—Treatment characteristics of the 2 patients with acute nonlymphocytic leukemia

Patient No.	Sex	Age (yrs)	Treatment date	Treatment No.	Volume infused	Infusion rate (ml/min)	Blast SIg (% cells positive)*
1	M	63		Baseline			17.7
			2/1/83	1	120 ml of serum	4	1.25
			2/4/83	2	120 ml of serum	4	8.9
			2/9/83	3	300 ml of serum	5	4.3
			2/18/83	4	400 ml of serum	6.5	
			2/22/83	5	300 ml of plasma	5	
2	F	41		Baseline			2.6
			2/11/83	1	150 ml of serum	2.5	14.7
			2/14/83	2	150 ml of serum	2.5	

* % of marrow leukemic blasts with surface immunoglobulin (SIg) was determined immediately before the next serum or plasma infusion.

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globulin declined moderately after treatment. On the other hand, an increase in leukemic blasts staining for surface immunoglobulin was observed in Patient 2 (table 1). Peripheral blood differentials did not reveal a consistent change in absolute lymphocyte counts or percentage of reactive forms.

DISCUSSION

Recent data suggest several possible mechanisms of action for the efficacy and toxicity of protein A-treated sera or plasma (4,5). Protein A may elute from collodion charcoal matrices or protein A-rich staphylococcal strains and then complex to immunoglobulin oligomers (4). Alternatively, anaphylatoxins (C3a, C4a, C5a) may be generated following perfusion over protein A (5). Previously, we showed that ^{125}I-protein A was not detected in serum which had been treated with ^{125}I-protein A-Sepharose in a manner identical to that used to treat our patients (3). Although we report only two patients, the absence of protein A in the treated sera may account for the lack of toxicity which previously has been observed after similar flow rates and quantities of infused sera or plasma. Therefore, further studies to define the toxicity and tumoricidal properties of infused protein A may be in order.

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Phase II Study of Mitomycin in Disseminated Malignant Melanoma[1]

E. T. Creagan,* J. H. Edmonson, D. L. Ahmann, and M. Chang[2]

Disseminated malignant melanoma remains discouragingly refractory to a spectrum of systemic investigational and conventional strategies. Dacarbazine and the nitrosoureas, the most extensively investigated agents, achieve response rates of approximately 20%, with median response durations of 2-4 months. In this clinical context, mitomycin seems appropriate for clinical trials.

An analog of mitomycin, an antitumor antibiotic, has documented activity against B16 melanoma (1). A broad phase II study involving 281 patients included 31 individuals with disseminated malignant melanoma. The objective response rate among 23 evaluable patients was 22% (2). Myelosuppression was the dose-limiting toxicity. We report this phase II study assessing mitomycin using an intermittent schedule (3) in patients with advanced malignant melanoma.

MATERIALS AND METHODS

Each patient had histologic proof of measurable or evaluable malignant melanoma, which was incurable by surgery or radiation therapy. Other criteria included wbc count $\geq 4100/mm^3$, plalelet count $\geq 130,000/mm^3$, serum creatinine ≤ 1.5 mg/dl, normal direct serum bilirubin, and Eastern Cooperative Oncology Group (ECOG) performance score (PS) of ≤ 3 (table 1).

Drug Administration

Mitomycin was administered iv into the tubing of a free-flowing infusion over 10-15 minutes at a dose of 15 mg/m^2 every 6 weeks.

Evaluation

Prior to initial treatment and subsequent courses, patients received a thorough general medical assessment, routine hematologic and chemical parameters, chest x-ray, and photographs of appropriate lesions.

An objective response was defined as a decrease of $\geq 50\%$ in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion in the absence of any new areas of malignant disease or symptomatic deterioration. Progressive disease was defined as a $> 25\%$ increase in any indicator lesion or the appearance of new lesions.

RESULTS

Therapeutic.—The response rate was 6% among 16 patients without prior systemic chemotherapy. Among 11 "good-risk" patients (no prior chemotherapy and PS = 0, 1), there was no objective regressions. Among 15 "poor-risk" patients (prior chemotherapy or PS = 2, 3), one experienced a complete response of soft tissue disease and a partial regression of a lung lesion for 2.7 months.

Toxicity.—Among 22 patients with wbc counts, one (4.5%) had a wbc count $\leq 1.5 \times 10^3/mm^3$. The median

TABLE 1.—Patient characteristics

Characteristics	No. of patients
Entered/evaluable	26
Median age in yrs (range): 54 (27-72)	
Sex	
Males	17
Females	9
ECOG PS	
0, 1	15
2, 3	11
Prior therapy	
Chemotherapy	10
No. of regimens	
1	3
≥ 2	7
Interferon	18
None	16
Indicator lesions	
Measurable	25
Evaluable	1
Dominant disease	
Visceral	15
Nonvisceral	11

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wbc count nadir was 4.0 × 10^3/mm^3 (range, 1.2-7.8). Among 21 patients with interim platelet counts, two (9.5%) had thrombocytopenia (< 75 × 10^3 cells/mm^3). The median platelet count was 140 × 10^3/mm^3 (range, 14-345). Mild to moderate nausea and vomiting affected 9% of 26 patients.

DISCUSSION

It seems improbable that mitomycin, in this dose and schedule, will meaningfully alter the presently inexorable course of disseminated melanoma. Our findings are consistent with other trials of mitomycin in this disease (4,5). However, it remains unclear if more aggressive treatment would have been beneficial in view of tolerable hematologic toxicity. No response among 11 good-risk patients is inconsistent at the 0.05 level, with a true response probability of ≥ 25%. One response among 16 patients without prior chemotherapy is inconsistent at the 0.05 level, with a true response probability of ≥ 27%. Thus, substantial antitumor activity of mitomycin is unlikely in patients with disseminated malignant melanoma.

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Phase II Trial of Carboplatin in Patients With Adenocarcinomas of the Upper Gastrointestinal Tract[1,2]

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Carboplatin (CBDCA) is a second-generation platinum analog designed to have a broader therapeutic index and less toxicity than cisplatin. CBDCA was shown to be active against a variety of transplanted murine tumor lines, including both leukemias and solid tumors (1). Several phase I trials have been performed; the dose-limiting toxic effect was thrombocytopenia (2,3). Cisplatin, when used as a single agent, has modest activity in previously treated patients with gastric cancer (4). The median duration of response was 5 months. We therefore conducted a phase II trial of CBDCA in patients with advanced adenocarcinomas of the upper gastrointestinal (UGI) tract to evaluate its activity against this disease and to further define its toxicity.

Twenty-seven patients with histologically proven adenocarcinomas of the UGI tract were entered in this study. All patients had measurable or evaluable disease. Measurable disease included well-defined masses in lymph nodes, subcutaneous tissue, or lung, or masses measurable by computerized tomographic scan. The primary tumor was considered to be evaluable by barium contrast studies; responses were independently quantitated by a reference radiologist (R. Heelan). Standard physiologic criteria for entry in a phase II trial were employed. Informed consent was obtained from all patients.

CBDCA was administered as a 1-hour iv infusion every 4 weeks. The initial dose was 400 mg/m^2, with dose escalation or attenuation according to the degree of myelosuppression. When severe thrombocytopenia was noted in previously treated patients, the starting dose for this subset of patients was decreased to 350 mg/m^2.

Patient characteristics are summarized in table 1. Sixty-five percent of the patients had received no prior chemotherapy or irradiation. Among the nine patients who had received prior chemotherapy, six had received only one regimen, two had received two regimens, and one had received three regimens. The most commonly used agents were 5-FU, doxorubicin, and mitomycin.

Of the 26 evaluable patients, 22 had disease progression, two had stable disease, and one had partial response (lasting 3 months). The observed response rate was 4% (95% confidence limits, 2%–18%). The responding patient had not received prior therapy.

The major toxic effect was thrombocytopenia. The median wbc count nadir was 3.4/mm^3 (range, 0.8–10.7). The median platelet count nadir was 106,000/mm^3 (range, 11,000–291,000). Among the 23 patients who received a dose of at least 400 mg/m^2, there were 35 evaluable courses, during which there were ten episodes (29%) of platelet counts < 100,000/mm^3. Ten courses of treatment were in previously treated patients; six had platelet counts < 50,000/mm^3. At the 350-mg/m^2 level, there were five evaluable courses, in three of which the platelet count nadir was

TABLE 1.—Pretreatment patient characteristics and study results

	No. of patients
Entered/evaluable	27/26
Median age in yrs (range): 58 (35–78)	
Median performance status: 70 (60–90)	
Males/females	20/7
Prior therapy	
None	17
Chemotherapy	8
Irradiation	2
Primary site	
Esophagus	1
Gastroesophageal junction	10
Stomach	16
Complete/partial response	0/1
Minor response/stable disease	1/2
Disease progression	22

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< 100,000/mm^3. Of the 47 courses of CBDCA, 11 (23%) were associated with platelet count nadirs < 50,000/mm^3. Three patients required platelet transfusions. Renal toxicity was not significant. Nausea and vomiting were mild and easily controllable with antiemetics.

DISCUSSION

Adenocarcinoma of the UGI tract remains a major therapeutic problem. In the presence of metastatic disease, median survival among nonresponding patients is usually 3–4 months in duration. CBDCA was chosen for phase II trial because cisplatin had shown activity against gastric cancer. Unfortunately, the analog has minimal activity in previously untreated patients with this tumor and no activity in those who had received prior chemotherapy. Our trial confirms the agent's toxicity of thrombocytopenia, especially in previously treated patients.

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Phase II Trial of Mitoxantrone in Refractory Germ Cell Tumors: A Trial of the Southeastern Cancer Study Group[1,2]

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Mitoxantrone (DHAD) is an anthraquinone antineoplastic agent with a wide spectrum of activity against animal tumor systems (1). Early phase II studies showed significant activity in breast cancer (2). Dose-limiting toxic effects are leukopenia and possibly cardiotoxicity in patients who have received previous anthracyclines (3). Phase I studies recommended doses of 12-14 mg/m^2 every 3 weeks or 5-6 mg/m^2/week for phase II evaluations (3). The Southeastern Cancer Study Group initiated this phase II trial in refractory germ cell tumors. A weekly schedule (5 mg/m^2) was chosen.

PATIENTS AND METHODS

Patients with advanced germ cell tumors progressing within 8 weeks of initial cisplatin-containing induction chemotherapy were eligible. Other patients were eligible after cisplatin plus etoposide salvage chemotherapy. All patients were to be unsuitable for surgical resection. Other requirements included adequate performance status, hepatic and renal functions, and no significant heart disease or extensive anthracycline therapy. A signed informed consent was required. Patients were to have either bidimensionally measurable disease or an elevated tumor marker (human chorionic gonadotropin or alpha-fetoprotein).

DHAD was given weekly by a 30-minute iv infusion at a dose of 5 mg/m^2. If no toxicity after six courses was observed, the dose was to be escalated every 2 weeks by 0.5 mg/m^2 to a maximum of 6 mg/m^2. The weekly dose was not given if the granulocyte count was $< 1400/mm^3$. Evaluable patients completed 6 weeks of study or 2 weeks if rapidly progressive disease had occurred. A partial response was defined as a 50% decrease in the sum of the products of the largest perpendicular diameters of all measurable disease. A complete remission was defined as the disappearance of all clinically detectable disease. For patients whose only evidence of disease was an elevated tumor marker, a 90% decline in this value was required for response. Disease progression was defined as a 50% increase in marker level.

RESULTS

Fourteen patients were entered between July 1982 and April 1984, all of whom were evaluable. The patient

TABLE 1.—Patient population, response, and toxicity

	No. of patients
Primary site	
Testis	11
Extragonadal	3
Karnofsky performance status	
80-100	9
70	4
60	1
No. of prior chemotherapy regimens	
1	3
2	5
3	3
4	1
6	1
8	1
Response parameters	
Bidimensionally measurable	13
Marker only	1
Prior doxorubicin	
Yes	4
No	10
Response	0
Hematologic toxicity	
Wbc count nadir (cells/mm^3)	
2000-3000	7
1000-2000	3
< 1000	1
Platelet count nadir (cells/mm^3)	
90,000-130,000	2
50,000-90,000	2
25,000-50,000	1

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population, response, and toxicity are described in table 1. They were heavily pretreated. All patients progressed on therapy, 12 prior to the 6-week evaluation. No patient had significant reduction in marker levels. The median number of courses given was three (range, one to six courses). Thirteen patients have died of disease. The median survival was 7.5 weeks.

The only common toxic effect was hematologic, which was comparatively mild. However, all patients experienced at least modest toxicity. There was no clinically evident cardiac damage or drug-related mortality.

DISCUSSION

No responses were seen in this heavily treated patient population (95% confidence limits, 0%–27%). These results are in agreement with a European Organization for Research on Treatment of Cancer study employing DHAD at a dose of 12–14 mg/m^2 every 3 weeks. No responses were seen in eight patients in this study (4). For all 22 patients, 95% confidence limits are 0%–18%.

It is conceivable that DHAD may have modest activity in less heavily pretreated patients, particularly if a higher dose was used, which should be feasible since toxicity in this study was relatively mild. However, considering the rarity of this disease and the results of these two studies, we do not feel further investigation of this agent is indicated.

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Phase II Evaluation of Cisplatin in Squamous Carcinoma of the Esophagus: A Southeastern Cancer Study Group Trial[1,2]

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Cisplatin is active in several types of cancer; our experience with this agent in esophageal cancer is reported here.

Adults with incurable squamous carcinoma of the esophagus were eligible if they had a measurable lesion; histologic proof of residual, recurrent, or metastatic carcinoma; a performance status of ≥ 50; a life expectancy > 6 weeks; recovered from surgery or previous chemotherapy; a serum creatinine of < 2 mg/dl; had not received prior therapy with cisplatin; and gave written informed consent. Cisplatin was to be given once every 21 days at a dose of 90 mg/m^2 by iv infusion at a rate of 1 mg/minute with adequate hydration. Toxicity was measured with standard criteria (1). A complete response was defined as complete disappearance of all objective evidence of cancer. A partial response was defined as a decrease of $\geq 50\%$ in the sum of the products of the diameters of measurable lesions and/or an increase of at least 50% in the width of the esophageal lumen and/or a reduction by 30% of the sum of the liver measurements below the costal margin in the case of hepatomegaly. No significant deterioration in weight or performance status was allowed for persons defined as having a response.

Of 29 patients entered into the study (table 1), one was considered ineligible because of a second malignancy, and a second was considered ineligible because of only limited disease. One patient was evaluable for toxicity alone. All patients received at least two courses of therapy. Of the 26 evaluable patients, one had a complete response and three had partial responses, for a total response rate of 15%. The duration of response was 1 month in the complete responder, and 1.5, 6, and 12 months, respectively, in the three partial responders.

Our results with cisplatin in esophageal cancer confirm the activity noted by others (2,3) but we could not confirm the 40% response rate observed in a pilot study with ten patients (4).

Current trials with cisplatin combinations are promising (5), but it should be recognized that cisplatin itself at 90 mg/m^2 is not highly active in esophageal cancer, and that improved results with combination chemotherapy will need to be confirmed in large-scale comparative trials.

TABLE 1.—Patient characteristics

Characteristics	No. of patients
Median age in yrs (range): 59 (46–84)	
Karnofsky performance status	
60	4
70	9
80	6
90	4
100	4
Not given	2
Sex	
Male	24
Female	5
Race	
White	12
Black	17
Prior treatment	
None	18
Radiation therapy	10
Radiation therapy and chemotherapy	1
Toxic effect (27 patients)	
Granulocytopenia < 1000 cells/mm^3	0
Nausea/vomiting	15
Renal	5
Neurologic	1

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Clinical Study of Patients With Cyclophosphamide-Induced Hemorrhagic Cystitis

A clinical study of the effect of mesna in preventing further hemorrhagic cystitis in patients treated with cyclophosphamide is being sponsored by Adria Laboratories. Eligible patients must have developed hemorrhagic cystitis from cyclophosphamide. The bleeding may not have been severe, ie, required blood transfusions or intravesical therapy. At the time of proposed further cyclophosphamide therapy they must have fewer than 50 RBC/HPF, no bleeding diathesis, and no bladder tumor. Adria Laboratories will supply mesna. Physicians will complete brief case report forms describing the patients and results of treatment.

For further information, contact: Jarid A. Simons, MD, PhD, Adria Laboratories, PO Box 16529, Columbis, OH 43216 (telephone: 614-764-8194) or call James K. Luce, MD (telephone: 614-764-8178).

Bristol-Myers Cancer Research Award

Nominations are now being accepted for the ninth annual Bristol-Myers Award for Distinguished Achievement in Cancer Research.

The award, a $50,000 prize and sterling silver medallion, is presented to a candidate chosen by an independent selection committee.

Nominations may be made by officers of medical schools, freestanding hospitals, and cancer research centers. Two letters of reference from individuals from outside the nominating institution must be provided.

The 1986 award selection committee will be chaired by Dr. Henry C. Pitot, Professor of Oncology and Pathology at the University of Wisconsin Medical School and director of its McArdle Laboratory for Cancer Research. The committee includes senior investigators from 12 institutions currently participating in the $8.34 million program of unrestricted grants for cancer research Bristol-Myers has sponsored since 1977.

For further information, contact: Kathryn R. Bloom, Manager, Public Affairs, Bristol-Myers Company, 345 Park Avenue, New York, NY 10154 (telephone: 212-546-4339).

Fellowship in Clinical Pharmacology

Institute Jules Bordet, Brussels, Belgium

A position for a clinical pharmacologist is open at the Institut Jules Bordet. The Institut Jules Bordet is a comprehensive cancer center, including medical, radiotherapeutic, and surgical disciplines. It has a strong commitment to preclinical and clinical research.

The medical department is presently expanding its pharmacologic unit and opening a position for a trained pharmacologist looking for an academic career. Candidates should have a strong background in preclinical and clinical pharmacology of antineoplastic agents and in screening and toxicologic programs. They should be familiar with high-pressure liquid chromatography, gas chromatography, atomic spectrophotometry, mass spectrometry, and other modern techniques in this field. Appropriate equipment and computer resources will be available. Close contacts with the clinicians will be necessary. An attractive salary will be proposed.

For further information, contact: J. Klastersky, MD, Institut Jules Bordet, 1, rue Heger-Bordet, B-1000 Brussels, Belgium (telephone: 32-2-537.33.28).

First Charles Heidelberger Pharmacology Seminar: Interface Between Cancer Pharmacology and Cell Biology

Los Angeles, California, January 13, 1986

The Comprehensive Cancer Center of the University of Southern California School of Medicine will sponsor this seminar that will cover areas of research that promise exciting new developments in cancer chemotherapy within the next decade. Topics include: biochemical modifiers; enzymes as targets for chemotherapy; methylphosphonate oligonucleotides; DNA-directed drugs; topoisomerase; gene amplification; and drug resistance.

There is open admission with no charge for attendance. Early registration is advised due to limited space.

For further information, contact: John Holcenberg, MD, c/o USC Comprehensive Cancer Center, 2025 Zonal Ave, Rm 508, Los Angeles, CA 90033.

Med Tech '86

Tampa, Florida, January 20–22, 1986

This conference is structured to address both the medical profession and hospital administrative organizations. It has been developed on two distinct tracts: (*a*) the International Symposium on Cancer Biology and Therapeutics (focusing on fundamental biological and biochemical aspects of the cancer cell and the current therapy of human malignancies which is derived from this basic knowledge) and (*b*) the International Healthcare Management and Technology Conference (to present the latest developments in computers, telecommunications, systems software, and support products/technology to serve the healthcare industry and to describe advances in management leadership within the fields of marketing, architecture, fiscal responsibility, and revenue generation under DRGs). A products and services exposition will also be featured.

The meetings will take place in the Curtis Hixon Convention Center in Tampa. Sponsors include: University of South Florida College of Medicine, Tampa; International Trade Fair Advisory Committee, City of Tampa; International Conference Management, Inc, Dallas, TX; and H. Lee Moffitt Hospital and Cancer Research Institute, USF Tampa. The Cancer Symposium is certified for 21 hours of credit (ACCME) in Category 1 for the Physician's Recognition Award of the American Medical Association; for 21 hours by the American Academy of Family Physicians; and for continuing education contact hours by the Florida Board of Nursing.

Poster session abstracts must be submitted by *December 20, 1985*, to: Joseph G. Cory, PhD, Program Director, Medical Center, Box 7, 12901 N 30th St, University of South Florida, Tampa, FL 33612. Mail registration will be accepted until *January 6, 1986*; thereafter, conference registration will be accepted only on-site.

For further information, contact: Colonel Mason, International Conference Management, Inc., 15851 Dallas Pkwy, Suite 1155, Dallas, TX 75248 (telephone: 214-458-7011).

Ninth Annual Course on Infectious Disease in Clinical Practice

Aspen, Colorado, January 25–February 1, 1986

The Extended Programs in Medical Education, University of California, San Francisco, is sponsoring this course on infectious disease in clinical practice which is scheduled to be held at the Conference Center, Snowmass at Aspen, Colorado. This course meets the criteria for 24 hours of AMA/CMA category I hours. Fees are: $375 for physicians, $325 for allied health, and $275 for interns and residents.

For further information, contact: Terrie Van Alen, Infectious Diseases Division, San Francisco General Hospital, 1001 Potrero Ave, 5H22, San Francisco, CA 94110 (telephone: 415-821-8200; office hours: T, W, Th, 8–11 AM).

The University of California, Irvine, First International Cancer Conference

Newport Beach, California, February 13–15, 1986

This 3-day conference, sponsored by the University of California, Irvine Cancer Center, The Orange County Unit of the American Cancer Society, and the Memorial Medical Center of Long Beach-University of California, Irvine, Center for Health Education, offers 22.5 hours of Category I credit, and will be held at the Newport Beach Marriott Hotel and Tennis Club.

For further information, contact: Assistant Director, Center for Health Education, 2801 Atlantic Ave, Long Beach, CA 90801 (telephone: 213-595-3823).

2nd Annual Conference on Decisions in Surgery

Snowmass, Colorado, March 1–8, 1986

The University of California, San Francisco, is sponsoring this conference on decisions in surgery as part of its Extended Programs in Medical Education. The conference is designed for general surgeons and addresses problems encountered in daily practice. It offers the opportunity to discuss pros and cons of various approaches and to formulate a rational basis for surgical decision-making. Topics for discussion will include: gastrointestinal surgery; pediatric and fetal surgery; thoracic surgery; trauma; sepsis; and biliary, esophageal, lung, and breast cancer.

This conference meets the criteria for 20 hours in Category 1 of the Physician's Recognition Award of the American Medical Association and the Certificate Program of the California Medical Association. Tuition is $400.00 ($475.00 for on-site registration), $200.00 for interns, residents, and medical students who submit a letter of verification from training program with application ($240.00 on-site).

For further information, contact: Extended Programs in Medical Education, Registration Office, 569-U, University of California, San Francisco, CA 94143 (telephone: 415-476-4251).

Annual Workshop of the San Francisco Academy of Hypnosis

San Francisco, California, March 1–2, 15–16, 1986

The San Francisco Academy of Hypnosis, an association of qualified physicians, dentists, psychiatrists, and psychologists, was formed to promote study and understanding of hypnosis as an adjunct to health care and treatment and to encourage research in the field of hypnotherapy. The academy will be giving its Annual Workshop in March of 1986.

The workshop, which will be held at the Letterman Army Medical Center, San Francisco, CA, will include basic theory, techniques, and applications of hypnosis as an adjunct treatment in health care with special sessions on application to each of the following specialties: stress management, pain control, habit change, as well as specific appropriate problems of medicine, dentistry, pediatrics, psychotherapy, and anesthesia.

CME credit offered will be 28 units of Category I. Fees are: $300, $150 for residents and interns, and $100 for students.

For further information, contact: Jan Brooks, San Francisco Academy of Hypnosis, 615 27th St, San Francisco, CA (telephone: 415-826-0533).

1986 Annual Meeting of the American Radium Society

San Francisco, California, April 6–10, 1986

For further information, contact: Ms. Suzanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

2nd International Symposium on the Surgery and Biology of the Greater Omentum

London, England, April 17–19, 1986

The symposium is to be held at the Royal Society of Medicine in London on April 17–19, 1986. The subjects to be covered are reconstructive surgery, immune responses, angiogenesis, peritoneal wound healing and adhesions, and omental tumor growth and metastasis. There will be free papers as well as lectures by invited speakers. Conference abstracts will be distributed at the beginning of the symposium and published later.

For further information, contact: Professor AJS Davies, Institute of Cancer Research, Royal Cancer Hospital, Chester Beatty Laboratories, Fulham Rd, London SW3 6JB, England.

First International Congress on Cancer and Hormones

Rome, Italy, April 23–27, 1986

This conference will include such topics as newer diagnostic and therapeutic approaches; epidemiology of endocrine-related cancer; hormone receptors and tumors; oncogenes, growth factors, and small effectors; hormones and cell proliferation; and cancers arising in endocrine glands.

For further information, contact: Stefano Iacobelli, MD, Laboratorio di Endocrinologia Molecolare, Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica del S. Cuore, L.go Agostino Gemelli, 8, 00168 Rome, Italy (telephone: (06) 33.054.496; telex: 611330 UNCATRO).

Fundamental Problems in Breast Cancer International Symposium

Banff, Alberta, Canada, April 27–29, 1986

The Breast Unit of the Cross Cancer Institute and the Division of Oncology, University of Alberta, are organizing this international symposium. Topics for discussion include: natural history of clinical significance of breast cancers detected by imaging techniques; endocrinology in growth and therapy of breast cancer; overview of important developments in breast cancer research; local and systemic therapy; radiological imaging techniques; future directions in therapy of breast cancer; and problems and priorities of cancer care and research in other countries.

This 3-day symposium will be held at the Banff Springs Hotel, Banff, Canada.

For further information, contact: Secretary, Breast Unit, Cross Cancer Institute, 11560 University Ave, Edmonton, Alberta, Canada T6G 1Z2.

International Symposium on Hormonal Manipulation of Cancer: Peptides, Growth Factors, and New (Anti)Steroidal Agents

Rotterdam, The Netherlands, June 4–6, 1986

This symposium is focused on new therapeutic approaches in the area of potential hormone-dependent tumors with respect to both peptide hormones and (anti)steroidal agents. Topics to be discussed are: LHRH-analogs; somatostatin-analogs; growth factors; peptide receptors, hyperprolactinemia; rescue or recruitment studies; new antisteroidal agents; new aromatase inhibitors; new applications for hormonal treatment; and alternating hormonal treatment. A free poster session will be included.

The symposium will be held in the Congress Center "De Doelen" in Rotterdam, The Netherlands. Abstract forms must be received *before March 1, 1986*.

For further information, contact: Congress Secretariat/Trial-and-Datadepartment, International Symposium "Hormonal Manipulation of Cancer," The Dr. Daniel den Hoed Cancer Center, PO Box 5201, 3008 AE Rotterdam, The Netherlands.

Fourth Annual European Organization for Cooperation in Cancer Prevention Studies (ECP) Symposium: Concepts and Theories in Carcinogenesis

Brugge, Belgium, June 12–13, 1986

This conference will be a combination of review lectures, discussion panels, and poster sessions. The following topics will be discussed: biochemistry of cell proliferation and differentiation; normal versus neoplastic tissue behavior, the multistep process from the normal cell to cancer; tumor/host adaptation-immune mechanisms; mechanisms in radiation carcinogenesis and chemical carcinogenesis; role of free radicals, oncogenes, and RNA viruses; DNA viruses and carcinogenesis; genetics and cancer; hormones and carcinogenesis; stress, aging, and cancer risks; multifactorial aspects in carcinogenesis; and the essential versus occasional aspects of carcinogenesis.

For further information, contact: ECP Secretariat, Avenue Lambeau 62, B-1200, Brussels, Belgium (telex: 62173 PRPB).

35th National Meeting of the Italian Neurosurgical Society

Pavia, Italy, September 18–20, 1986

Topics of discussion at this meeting include cerebral aneurysms, pathology of the cervical column, brain tumors in infancy, spinal tumors, cerebral ischemia, and pain therapy.

For further information, contact: Prof. Pietro Paoletti, Department of Surgery, Neurosurgical Clinic, University of Pavia, San Matteo Hospital, 27100 Pavia, Italy (telephone: 0382/422231).

Second International Symposium on Epstein-Barr Virus

St. Petersburg, Florida, October 17–22, 1986

This symposium, dedicated to the memory of Professor Yohie Ito, will cover the current advances in Epstein-Barr Virus (EBV)-associated diseases such as Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative syndrome, chronic infectious mononucleosis, and immunodeficiency syndromes including AIDS and organ transplants. The various scientific presentations will include overviews, selected papers, and poster sessions. The program will concentrate on both clinical and basic research aspects. Specifically, the symposium will review the latest advances in disease manifestations associated with EBV infections and will cover clinico-pathologic features, epidemiologic and genetic aspects, and new developments in basic research on EBV including: interaction of oncogenes, EBV genomes, gene expressions, mapping and proteins, cellular immune responses, co-factors associated with EBV infections, and control of EBV infections.

Dr. M. Nonoyama, Showa University Research Institute for Biomedicine in Florida; Dr. R. Glaser, Ohio State University; Dr. G. Pearson, Georgetown University; and Drs. D. Ablashi and P. Levine, National Cancer Institute, Bethesda, MD, are organizing the symposium.

For further information, contact: Dr. Nonoyama, Showa University Research Institute for Biomedicine in Florida, St. Petersburg, FL 33702; or Dr. Ablashi, Bldg 37, Rm 1E24, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (telephone: 301-496-9332).

Cancer Treatment Symposia: Proceedings of the Workshop on Radiotherapy for Lung Cancer

The International Cancer Information Center of the National Cancer Institute announces the availability of the Proceedings of the Workshop on Radiotherapy for Lung Cancer, which focused on important unsolved problems in this type of cancer therapy. Held at King's College, Cambridge, England, on June 26–30, 1984, the workshop was sponsored by the International Association for the Study of Lung Cancer and the International Society for Radiation Oncology.

At this workshop, leading investigators discussed the results of current clinical studies and the definition of areas suitable for future research in the management of both small cell and non-small cell bronchial carcinoma. Topics include the role of radiation therapy in combined-modality treatment plans; the effect of radiation in controlling both the primary lesion and central nervous system disease in small cell carcinoma; quality control of radiotherapy; and the relative efficacy of radiation therapy and systemic chemotherapy.

Copies of this volume of *Cancer Treatment Symposia* (Volume 2, 1985) are available for $8.50 from the US Government Printing Office. To order, use the form below. Make checks payable to the Superintendent of Documents and mail to the address on the order blank.

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